

Antipsychotic Drugs: Importance of Dopamine Receptors for Mechanisms of Therapeutic Actions and Side Effects

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Abstract—Interaction of the antipsychotic drugs with dopamine receptors of the D₂, D₃, or D₄ subclasses is thought to be important for their mechanisms of action. Consideration of carefully defined affinities of the drugs for these three receptors suggests that occupancy of the D₄ subclass is not mandatory for achieving antipsychotic effects, but actions at D₂ or D₃ receptors may be important. A major difference between typical and atypical antipsychotic drugs is in the production of extrapyramidal side effects by the typical drugs. Production of extrapyramidal side effects by typical drugs seems to be due to the use of the drugs at doses where striatal D₂ receptor occupancy exceeds ~80%. Use of these drugs at doses that do not produce this level of receptor blockade enables them to be

used therapeutically without producing these side effects. The antipsychotic drugs have been shown to act as inverse agonists at D₂ and D₃ dopamine receptors, and this property may be important for the antipsychotic effects of the drugs. It is suggested that the property of inverse agonism leads to a receptor up-regulation upon prolonged treatment, and this alters the properties of dopamine synapses. Several variants of the dopamine receptors exist with different DNA sequences and in some cases different amino acid sequences. These variants may have different properties that alter the effects of dopamine and the antipsychotic drugs. The determination of such variants in patients may help in the prediction of drug responsiveness.

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I. Introduction

The antipsychotic drugs are used very widely to treat the positive symptoms of schizophrenia, with some of the newer drugs having effects on negative symptoms, but there is still much debate about their precise mechanism of action. Thought in the area has been dominated by the now classical observation that the potency of antipsychotics to bind to the pharmacologically defined D_2 dopamine receptor correlated over a wide range of drugs, with the typical daily clinical dose of the drugs for the treatment of schizophrenia (Creese et al., 1976; Seeman et al., 1976). No such correlation of the daily dose was seen with the potencies of the drugs at other receptors, including D_1 dopamine receptors. These observations were made before the identification of multiple dopamine receptor subtypes by gene cloning, which showed that these actions could be at D_2 , D_3 , or D_4 receptors (the D_2 -like receptors) (for a review, see Neve and Neve, 1997). It is now important to ask certain questions about the mechanisms of these drugs, and I wish to address some of these questions.

II. Which Dopamine Receptor Isoform Is Important for Antipsychotic Action?

To answer this question, it would be desirable to calculate the occupancies of the different D_2 -like receptors by typically used doses of antipsychotic drugs. Here, we need accurate values for the dissociation constants of the drugs at the different receptor subtypes, and these can, in principle, be obtained using in vitro competition binding assays versus a suitable radioligand. There is considerable variation in values for dissociation constants for these drugs (K_i) in the literature. For example, in one publication, values for K_i for haloperidol of between 0.47 and 9.6 nM were reported using different radioligands and tissue sources (Seeman and Van Tol, 1995). Much of this variation results from technical problems in the ligand binding assays used, such as lack of equilibration and depletion of the radioligand concentration (for a more detailed discussion, see Strange, 1997), and it is worthwhile considering the problems.

A. Problems with Ligand Binding Assays for D_2 Dopamine Receptors

Many studies have been published on the binding of drugs to D_2 dopamine receptors and valuable data on drug affinities for the receptors have been accumulated in this way. To use these data in a quantitative manner it is necessary to be aware of certain problems in the use of some of the more popular radioligands. These problems can also extend to the use of these radioligands for in vivo scanning. The principal method for the determination of drug affinities is the competition ligand binding assay, and many studies have used the high-affinity radioligands [3 H]spiperone or [3 H]nemonapride. To obtain accurate estimates of dissociation constants for

competitors, however, it is essential to have accurate estimates of the dissociation constants (K_d) for the radioligands. Some variation in these may be attributed to the use of different assay conditions (e.g., use of different buffers by different laboratories). Other problems may arise in the use of these radioligands, and these concern mostly the lack of equilibration of radioligands and competitors with the receptor and the depletion of the ligands by binding to receptor or tissue. These problems have been recognized for some time and have been comprehensively reviewed (Chang et al., 1975; Golds et al., 1980; Wells et al., 1980; Burgisser et al., 1981; Seeman et al., 1984; Hulme and Birdsall, 1992; Strange, 1997) but are often ignored.

The problems are particularly acute for [3 H]spiperone and [3 H]nemonapride because these radioligands have rather low K_d values (~ 20 pM), when determined accurately (Hoare and Strange, 1996; Malmberg et al., 1996). [125 I]Epidepride may suffer from similar problems (Joyce et al., 1991). These low K_d values mean that, in a saturation radioligand binding experiment, radioligand concentrations above and below this value must be used. Equilibration of the radioligands with the receptors depends on the association and dissociation rates and the radioligand concentrations. At the low radioligand concentrations, the approach to equilibrium may be limited by the dissociation rate, and for high-affinity radioligands this may lead to some lack of equilibration and an overestimation of the K_d . Incubation times should therefore be extended beyond the typically used 1 h (25°C) for these radioligands.

Depletion of added ligands may also be a problem in that it confounds the definition of the actual free ligand concentration that is required for the application of equations defining binding equilibria. Depletion can occur by binding to receptors in the assay or by binding to tissue in a non-specific manner that disturbs the equilibrium. Corrections can, however, be made for the depletion due to binding of radioligands to receptors, but the nonspecific binding to tissue cannot be assessed accurately in a filtration assay. Where depletion is high, any corrections will be inaccurate, so the only way to avoid these problems is to work under conditions that avoid or minimize depletion. This requires either very low tissue concentrations or a radioligand that is not sensitive to these problems. I have discussed the quantitative aspects of these issues elsewhere (Strange, 1997) but if the high-affinity radioligands [3 H]spiperone and [3 H]nemonapride are being used, then accurate values of K_d will only be obtained under typically used conditions (~ 20 pM receptor) if large assay volumes (~ 10 ml) are used to dilute the tissue to reduce depletion of the radioligand in a saturation assay (extended incubation times will also be required as considered previously).

Alternatively, a lower affinity radioligand such as [3 H]raclopride ($K_d \sim 1$ nM) can be used where depletion is less important at this receptor concentration (higher radioligand concentrations will be used), and problems

with equilibration are absent (the dissociation rate constant is higher). The availability of receptors expressed at high levels in recombinant systems, has, however, increased the likelihood of depletion artifacts arising from binding to receptors as higher levels of receptor are more readily available.

These problems are also present in competition assays and should not be ignored, although because the radioligand concentrations used are usually higher, depletion problems are often less important. Equilibration problems may occur in competition assays, however, where the presence of the competitor slows down the approach to equilibrium of the radioligand (see Motulsky and Mahan, 1984). Importantly, however, the correction of IC_{50} values from competition data for the radioligand concentration requires accurate values of K_d for the radioligand, and if these are inaccurate (see above) then so are the derived K_i values for competitors. Once these problems are taken account of and accurate K_d values for radioligands are derived, then the accurate K_i values for competitors can be derived and these are similar irrespective of the radioligand used. In Table 1, I have given some values for K_i for antipsychotic drugs that take these considerations into account. It has been proposed (Seeman and Van Tol, 1995) that there is a relation between the "tissue-buffer partition coefficient" for the radioligand and the K_i for a competing drug in these assays. Extrapolation of the relation to zero partition gives a "radioligand-independent dissociation constant", but, this has no theoretical basis and indeed the use of the correct K_d value in the correction of IC_{50} values should yield a "radioligand-independent dissociation constant" anyway.

When these considerations are taken account of, it is clear that there are large discrepancies between the

actual K_i values for drugs at the D_2 dopamine receptor and those commonly reported in the literature. The discrepancy depends largely on the discrepancy in K_d values found for [3H]spiperone. There are many papers where the K_d for this radioligand is reported as being in excess of 100 pM, and this will lead to at least a 5-fold discrepancy in derived K_i values. This becomes very important if the K_i values for drugs are being used to infer conclusions about the specificity of different receptor subtypes. For example, it has been claimed that clozapine is a selective D_4 receptor antagonist (Van Tol et al., 1991). Examination of the K_i values in Table 1 shows that the selectivity is low and had been overestimated previously owing to overestimation of the K_i at the D_2 receptor when determined in competition versus [3H]spiperone.

Problems with the use of high-affinity ligands can also be seen in the in vivo scanning techniques. For example, some of the earlier studies used ligands related to spiperone (e.g., [^{11}C]N-methylspiperone). The binding properties of these ligands are such that they do not reach equilibrium at the receptors during the scan, and this leads to problems in the interpretation of experiments that will be considered later.

B. Comparison of Affinities for Antipsychotic Drugs at Different D_2 -Like Receptor Subtypes

Examination of the values for K_i shows that the affinity of the substituted benzamide drugs for the D_4 receptor is low. Given that these drugs are effective antipsychotics, this suggests that occupancy of the D_4 receptor may not be mandatory for the antipsychotic therapeutic effect. L745870 has been synthesized and is selective for the D_4 receptor [K_i (nM) values: $D_2 = 960$, $D_3 = 2300$, $D_4 = 0.43$ (Patel et al., 1997)]; it should be noted that these values have not been determined under optimal conditions and may be slight underestimates of potency at the D_2 receptor]. In a clinical trial with L745870, however, no antipsychotic activity was seen (Bristow et al., 1997). Although not much is known about the pharmacodynamics and pharmacokinetics of the drug in question, this observation is consistent with the view that the D_4 receptor does not play a major role in the antipsychotic actions of the other drugs. This then leaves the D_2 and D_3 receptors as potential sites of action of antipsychotic drugs. Indeed, it may be that both of these subtypes may need to be occupied to achieve antipsychotic action. This would be consistent with the complexity of schizophrenia and the multiple neuronal systems that are probably involved. In principle it should be possible to calculate the occupancy of the D_2 and D_3 receptors using the dissociation constants in Table 1. This is, however, a difficult enterprise, because it requires that we know the concentration of drug at the receptors and the concentration of dopamine in the synapse and neither of these quantities is easily defined. One approach to this problem has used the free drug

TABLE 1
Dissociation constants of antipsychotic drugs at dopamine receptor subtypes

	K_i (nM)		
	D_2	D_3	D_4
Chlorpromazine	0.55	1.2	9.7
Clozapine	35	83	22
Haloperidol	0.53	2.7	2.3
Olanzapine	7.5	49	15
Quetiapine	105	340	2000
Raclopride	1	1.8	2400
Remoxipride	54	969	2800
Risperidone	1.3	6.7	7.5
Sertindole	0.38	1.6	10.1
(-)-Sulpiride	2.5	8	1000
Thioridazine	1.2	2.3	10

Values given for the $D_{2(short)}$ and D_3 receptors are mostly taken from Malmberg et al. (1993), Seeman (1996), and Seeman and Van Tol (1995), and are from competition experiments versus [3H]raclopride binding to the receptors expressed in CHO cells. Values for olanzapine and quetiapine for the D_3 receptor are from Schotte et al. (1996) in competition experiments versus [^{125}I]iodosulpride. [3H]Raclopride and [^{125}I]iodosulpride are radioligands that can be used under conditions that avoid experimental artifacts (see text). For the D_4 receptor, data are taken from Seeman (1996), Seeman and Van Tol (1994), and Seeman and Van Tol (1995) using competition versus [3H]spiperone. These values must be taken as estimates only, because it is unclear whether the binding of [3H]spiperone at the D_4 receptor suffers from the same artifacts as does its binding to the D_2 receptor (see Section II).

concentration in plasma water for the calculations (Farde et al., 1989; Seeman, 1992), but it has been shown in rats that antipsychotic drugs are concentrated in the brain relative to serum and this concentration occurs to different extents for different drugs (Tsuneizumi et al., 1992; Baldessarini et al., 1993). For example clozapine, thioridazine, and haloperidol are concentrated, respectively, 24-, 1.4-, and 22-fold over the plasma level so that the relative plasma concentrations of the drugs are a poor guide to the relative free drug concentrations in the brain.

It is also necessary to know the concentration of dopamine in the synapse with which the antipsychotic is competing for access to the dopamine receptors. The dopamine concentration at the synapse has been determined to be ~200 nM for the striatum, but with a very steep concentration gradient decreasing away from the synapse (Kawagoe et al., 1992). Values for the extrasynaptic dopamine concentration vary between 6 and 89 nM (Sharp et al., 1986; Kawagoe et al., 1992; Garriss and Wightman, 1994). It has been shown that D₂ receptors can be located both synaptically and extrasynaptically (Yung et al., 1995). Because of the difficulties in defining the drug concentration and the dopamine concentration at the synapse, it is very difficult to calculate the actual occupancies in this way.

C. Use of Radiolabeled Antipsychotic Drugs to Image Dopamine Synapses In Vivo

Imaging studies can also be used to infer some of the properties of dopamine synapses in vivo in nonhuman primate and human brain. Amphetamine administration has been shown in two studies to increase dopamine levels in the striatum in nonhuman primates (Breier et al., 1997; Laruelle et al., 1997b) and in humans (Laruelle and Abi-Dargham, 1999) and SPECT² or PET scanning has been used to show that this increase in dopamine reduces in vivo striatal [¹²³I]IBZM or [¹¹C]raclopride binding. In one of these studies (Laruelle et al., 1997b), treatment of the animals with the dopamine synthesis inhibitor α MPT was found to reduce levels of dopamine and increase in vivo [¹²³I]IBZM binding. A similar study has also been performed in humans (Laruelle et al., 1997a). Tsukada et al. (1999) have also examined the effects of amphetamine and the dopamine transporter inhibitor GBR 12909 using [¹¹C]raclopride binding, and Ginovart et al. (1997) have examined dopamine depletion following treatment with reserpine in nonhuman primates.

In the studies with amphetamine, it seems that the dopamine released by the amphetamine competes with the radiolabeled tracer and reduces its binding. If it is assumed that dopamine and tracer compete and come to equilibrium at a uniform population of receptors, all of

which are accessible during the experiment, then the equations under *Section VI.C.* can be used to define the fractional occupancy of the receptors before and after amphetamine administration. Data of Breier et al. (1997) imply a fractional occupancy of ~0.03 by dopamine before amphetamine administration, whereas those of Laruelle et al. (1997b) imply a fractional occupancy of ~0.05. In the study of Tsukada et al. (1999), a baseline occupancy of ~0.01 is implied. Following amphetamine administration, occupancy values by dopamine increase to 0.3–0.4 for the highest amphetamine concentrations used. The baseline occupancy values in these studies are rather low (0.01–0.05), and this may imply that the in vivo scanning techniques are identifying changes in the occupancy of extrasynaptic receptors. In Laruelle et al. (1997b) and Tsukada et al. (1999), the baseline dopamine level was determined (~12 nM and ~6 nM, respectively), and these are more in line with the values for extrasynaptic dopamine given previously. If we use the baseline occupancy values and the measured dopamine concentration, then a dissociation constant of 220–600 nM is implied. This is in reasonable agreement with the value for the affinity of dopamine for the D₂ receptor determined in the in vitro ligand binding experiments in the presence of GTP (~1 μ M; Neve and Neve, 1997), which is thought to represent binding to the free receptor uncoupled from the G protein.

There has been much discussion about the appropriate affinity to use for dopamine in these studies (for example, see Fisher et al., 1995; Laruelle, 2000). In the in vitro experiments in the absence of added guanine nucleotides dopamine binding to D₂ receptors seems to be to two states of higher and lower affinity (Gardner et al., 1997). These higher and lower affinity states result from the coupling of the D₂ receptors to G proteins. In the presence of GTP, however, a single lower affinity state is seen for dopamine, which is thought to correspond to receptor uncoupled from G protein. Similarly, in whole cells where there are sufficient guanine nucleotides to uncouple receptor and G protein, a single affinity state is seen for dopamine (for example, see Sibley et al., 1983). It seems likely, therefore, that in imaging studies on intact brain, the binding of dopamine will be to this lower affinity state. This is not to say that the receptor does not couple to G proteins under these conditions, it is just that the coupled state that results in the appearance of the higher affinity dopamine binding state forms and breaks down rapidly if there are high concentrations of guanine nucleotides present.

The data of Laruelle et al. (1997b) with α MPT show an increase of 30% in [¹²³I]IBZM binding after dopamine depletion, and this is associated with a 50% reduction in baseline dopamine levels. These data imply a substantial baseline occupancy of receptors by dopamine. If we assume that α MPT reduces synaptic and extrasynaptic dopamine in proportion, then these changes in tracer binding may be used with the equations under *Section*

² Abbreviations: SPECT, single-photon emission computed tomography; PET, positron emission tomography; α MPT, α -methyl-para-tyrosine; IBZM, (S)-(-)-3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide.

VI.C. to estimate the fractional baseline occupancy by dopamine as 0.46. This is very different from the value inferred from the amphetamine experiments and implies a much higher level of dopamine, assuming the properties of the receptors are similar. In a further study in humans (Laruelle et al., 1997a), α MPT treatment lead to a 28% increase in [123 I]IBZM binding. Dopamine depletion was estimated to be 70%, and this implies a fractional baseline occupancy of 0.63. Using reserpine to deplete dopamine, Ginovart et al. (1997) showed that [11 C]raclopride binding increased and that this was due to a change in the K_d of the tracer, not a change in the number of binding sites. These observations would be expected if the changes in tracer binding result from a competitive interaction between dopamine and the tracer in the brain. The data of this study imply a baseline occupancy by dopamine of ~ 0.35 .

The data obtained using in vivo scanning and either dopamine release or dopamine depletion provide very different estimates of the baseline dopamine occupancy of D_2 receptors. One way to reconcile these observations is to propose that, depending on the conditions used, the observed [123 I]IBZM binding is to synaptic or extrasynaptic receptors or to both. The dopamine concentration is lower at the extrasynaptic receptors leading to a low occupancy by dopamine (<0.05), whereas at the synaptic receptors the dopamine concentration is higher and the occupancy correspondingly higher (~ 0.5). The baseline occupancy of receptors by dopamine synaptically (~ 0.5) is consistent with inferred synaptic dopamine levels (~ 200 nM; Kawagoe et al., 1992) and the K_d inferred previously. This analysis of tracer binding into separate pools of synaptic and extrasynaptic receptors in the two kinds of experiment is, however, an over simplification and given the present level of information it is not possible to be sure about the relative sizes of the labeled pools. We can say, however, that the dependence of the changes in tracer binding on changes in dopamine after amphetamine and after α MPT are different and consistent with low and high starting dopamine occupancies, respectively. It seems likely that, after α MPT treatment, the changes in tracer binding are largely synaptic, because whether extrasynaptic dopamine is low then a further lowering of this will have little effect. It seems likely, also, that the majority of the change in tracer binding seen after amphetamine is extrasynaptic. It may be relevant that release of dopamine in the striatum has been shown to occur from synaptic and nonsynaptic sites (see, for example, Moore et al., 1999). Synaptic dopamine concentrations are high and phasic, whereas extrasynaptic dopamine concentrations are lower and tonic. These two pools of dopamine may be related to the different results obtained in the in vivo scanning experiments.

Laruelle (2000) has provided an extensive analysis of these imaging studies and their implications. He has suggested that the changes in tracer binding observed

after amphetamine administration are synaptic, in contrast to the conclusions reached previously. Further experimentation is required to resolve these differences. He also highlights an important problem with the studies that relates to the time course of the changes in tracer binding after, for example, administration of amphetamine. In several studies, the reduction in tracer binding following amphetamine is prolonged, and this is inconsistent with the kind of competitive models assumed here and in Laruelle (2000). Whether this is a reflection of receptor internalization, as suggested by Laruelle (2000) remains to be seen.

The technique of monitoring changes in tracer antipsychotic binding after manipulation of dopamine levels has been used in several interesting physiological situations. Piccini et al., 1999 studied dopamine release from unilateral nigral implants (in to the right putamen) in a patient with Parkinson's disease. [11 C]raclopride binding was reduced by 27% on the grafted side following amphetamine administration whereas there was only a small response (4%) on the nongrafted side. This shows that the implant is functional in terms of dopamine release.

Koepp et al. (1998) used [11 C]raclopride binding to study dopamine release in the striatum during the performance of a video game, as a measure of motor function. A 13% decrease in [11 C]raclopride binding was seen during the performance of the game and based on the figures in Table 4. This implies an increase of ~ 5 fold in extrasynaptic dopamine. This is an important demonstration of the extent of dopamine release during human neuronal function.

The technique has been used to examine the release of dopamine in the striatum in schizophrenic patients (Breier et al., 1997; Laruelle et al., 1999; Laruelle and Abi-Dargham, 1999), and these studies showed that following amphetamine administration there is a greater release of dopamine in schizophrenics as compared with normals [17% and 8% reduction of tracer binding, respectively, in the largest study (Laruelle et al., 1999)]. These figures imply increases of ~ 8 - and ~ 4 -fold in extrasynaptic dopamine in schizophrenics and normal patients, respectively. The increased dopamine release was only seen in patients suffering an episode of clinical deterioration, but not in clinically stable patients implying that the increased dopamine release is associated with the psychotic symptoms but not the underlying disease. Further work has provided evidence for an increased baseline occupancy of receptors by dopamine in schizophrenia using the α MPT dopamine depletion technique (Abi-Dargham et al., 2000)

D. Calculation of Dopamine Receptor Occupancies Using in Vivo Scanning Techniques

One way to get around the problems in the calculation of receptor occupancies by the antipsychotic drugs is to use data from imaging techniques (PET and SPECT).

Here, a tracer (radioactive drug) is used to label the receptors in human brain, and the occupancy of the receptors by an administered drug is determined from the reduction in tracer occupancy. These techniques then provide actual occupancy values for the drugs at the receptors. Data for the occupancy of striatal D_2 receptors by a range of drugs can then be used to calculate the actual drug concentration (using the relevant K_i value), and these concentrations can be used to infer occupancies of the D_3 and D_4 receptors. These occupancy data are given in Table 2 together with the concentrations of drugs inferred. These data emphasize the conclusion reached earlier that occupancy of the D_4 receptor is not mandatory for antipsychotic drug action, but it seems that occupancy of both D_2 and D_3 receptors could be occurring. To determine which receptor subtype (D_2 , D_3) is important for antipsychotic action, it will be necessary to identify selective agents for the two subtypes and test these in humans. Some progress is being made at producing these selective agents (Whetzel et al., 1997). A second observation that can be made about the data of Table 2 is that, for some of the atypical antipsychotic drugs (clozapine, olanzapine, and quetiapine), their affinities for D_2 and D_3 receptors are quite low. This will be discussed below.

Calculations of this kind, however, assume a uniform drug concentration within the brain, and they do not take account of differences in the levels of dopamine in different brain regions or differences in its affinity at the different receptor subtypes. Extracellular dopamine concentrations have been determined in different brain regions in the rat, and the concentration is very similar in caudate/putamen and nucleus accumbens but ~5- to 10-fold lower in frontal cortex (Sharp et al., 1986; Garriss and Wightman, 1994). The figures are shown in Table 3, and it is clear that the tissue content of catecholamine is a very poor guide to the available dopamine. In *Section VI.A*, I have given a derivation of the equations for the three-way competition of tracer, drug, and dopamine at the synapse, and this shows that if functional dopamine

TABLE 3
Extracellular dopamine concentrations and dopamine content in different brain regions

	Caudate/ Putamen	Nucleus Accumbens	Frontal Cortex
Extracellular dopamine (nM)			
Garris and Wightman, 1994	89	68	11
Sharp et al., 1986	50	30	6
Dopamine content (ng/mg protein)			
Garris and Wightman, 1994	90	90	1

levels are different in human tissues, as they are in the rat, then the occupancies by antipsychotic drugs may be higher in the tissue with the lower dopamine (e.g., cerebral cortex). The effects, however, will depend on the actual occupancy by dopamine in the different tissues and some possibilities are outlined under *Appendix*. The occupancies of D_2 -like dopamine receptors by antipsychotic drugs in the striatum and temporal cortex have been reported using SPECT and PET scanning, and greater occupancies are seen with the atypical drugs clozapine, olanzapine, quetiapine, and sertindole in cortical regions (Pilowsky et al., 1997; Meltzer et al., 1999; Bigliani et al., 2000; Stephenson et al., 2000), whereas with typical drugs no significant difference in occupancy was recorded (Bigliani et al., 1999). A study of one patient with clozapine failed to replicate these differential occupancies in striatal and cortical regions (Farde et al., 1997). This will be discussed further.

III. What Is the Mechanistic Basis for the Difference Between Typical and Atypical Antipsychotics?

Several antipsychotic drugs, including clozapine, olanzapine, quetiapine, and risperidone, are often referred to as the atypical antipsychotics in contrast to the other drugs that are referred to as typical antipsychotics. There has been some debate about the definition of typical and atypical antipsychotics and whether this is a quantitative or qualitative distinction (Kane, 1997; Waddington and O'Callaghan, 1997; Gerlach, 2000). One key difference is that the typical drugs cause some extrapyramidal side effects, whereas the atypical drugs generally do not; in terms of drug design, it would be of great use to understand this difference. It is generally assumed that the therapeutic antipsychotic actions of the drugs are mediated at dopamine (D_2 , D_3 ; see above) receptors in limbic or cortical regions, whereas the extrapyramidal side effects are mediated via striatal dopamine receptors (see, for example, Lidow et al., 1998). The extrapyramidal side effects will be mediated by striatal D_2 receptors, because these are the predominant D_2 -like receptors found in this tissue. The atypical drugs could, therefore, be having selective effects on cortical/limbic dopamine receptors with a minimal blockade of striatal dopamine receptors, or some additional feature of the drug could lead to a suppression of the extrapyramidal side effects and this could differ for

TABLE 2
Occupancies of brain dopamine receptors by antipsychotic drugs

Drug	Percent Occupancy			Drug Concentration
	D_2	D_3	D_4	
				nM
Chlorpromazine	78	62	17	1.95
Clozapine	38–63	21–42	49–73	21.5–59.6
Haloperidol	85	52	57	3.0
Olanzapine	43–89	10–55	27–80	5.6–60.7
Quetiapine	51	24	88	109
Raclopride	80	69	0.2	4
Risperidone	63–89	25–61	22–55	2.2–10.5
Sulpiride	78	53	1	8.9

Receptor occupancies for the D_3 receptor in the striatum were taken from PET scan data in Farde et al (1992), Nordstrom et al. (1995), Hagberg et al. (1998), and Kapur et al. (1999), and were used to calculate concentration of the drug in the brain using the dissociation constants from Table 1. Drug concentration was then used to calculate the percent occupancy of D_3 and D_4 receptors using their dissociation constants in Table 1.

different drugs (see, for example, Arnt and Skarsfeldt, 1998; Remington and Kapur, 2000). There are other attributes that differentiate typical and some atypical antipsychotic drugs, e.g., ability to treat patients resistant to other antipsychotics, efficacy on negative symptoms, but I shall restrict the subsequent discussion to the origins of the extrapyramidal side effects.

A. Blockade of Dopamine Receptors Achieved by Antipsychotic Drugs

Let us consider the effects of the antipsychotic drugs on dopamine systems and the proposition that the differences between typical and atypical antipsychotics with respect to the occurrence of extrapyramidal side effects reside largely in differential effects on cortical/limbic and striatal dopamine systems. Here, we need to consider not just the binding of these drugs to the receptors but the manner in which they interfere with the synaptic actions of dopamine. In *Section VI.B.*, I have derived the equations that apply to these effects. Based on these equations, the important conclusion is that, assuming that equilibrium is achieved between drug and dopamine at the receptors (see below), the effects of a drug that blocks dopamine action will depend on the ratio of its synaptic concentration (A) to its dissociation constant at the receptor (K_A), i.e., the A/K_A ratio. Drugs with low K_A are usually used at lower doses, whereas drugs with higher K_A are used at higher doses, therefore the A/K_A ratio will be similar. It has been argued recently that the atypical drugs are atypical because they compete less well with synaptic dopamine (Seeman and Tallerico, 1998). This is unlikely to be the case if the drug and dopamine reach equilibrium, and the A/K_A ratio is maintained for the different drugs as it superficially seems to be. This argument is complicated by the differences in drug levels achieved in the brain (see above and below), and here we are back to the problem encountered in *Section I* of accurately defining the synaptic concentration of a drug. Also, the interactions of dopamine and the antipsychotic drugs with the receptor may not be at equilibrium (see *Section III.B.*). Nevertheless, let us consider the results of some of the more recent analyses of PET studies on the effects of antipsychotic drugs.

In the early work, analyzing the occupancies of striatal D_2 receptors by antipsychotic drugs using PET scans, the occupancies determined for typical antipsychotic drugs were 70% or more (Table 2), and it was shown that if occupancy exceeded about 80% then extrapyramidal side effects were seen (Farde et al., 1992; Nordstrom et al., 1993). It was suggested that different mechanisms might be mediating the therapeutic and side effects and that different receptor occupancies were involved. Indeed, lower occupancies were reported for clozapine (~60% or less), and this might account for the lack of extrapyramidal side effects seen with this drug. More recently, work has been performed with the typical drug

haloperidol, and it has been shown that using lower doses of this drug in its decanoate form yields a good clinical response without side effects, and the D_2 receptor occupancy is only about 50% (Nyberg et al., 1995). This supported an earlier clinical study (McEvoy et al., 1991) showing that low doses of haloperidol gave satisfactory clinical effects and that increasing the dose only lead to a greater incidence of extrapyramidal side effects. The occupancy of D_2 receptors during antipsychotic therapy has been examined in a careful study with haloperidol (Kapur et al., 2000). Using low-dose haloperidol (1–2.5 mg/day) substantial intersubject variation in D_2 receptor occupancy was seen (39–87%), but clinical response was achieved with a D_2 occupancy greater than 65%, whereas extrapyramidal side effects were seen if D_2 occupancy exceeded 78%. This emphasizes the narrow dose range in which clinical response is seen without side effects for this drug. A study with clozapine has shown that there is great variability in both the plasma concentration of drug achieved and the occupancy of D_2 receptors, despite a good clinical response being seen (Pickar et al., 1996). In that study, it was suggested that there might be trait-like variation in the clinical response to clozapine. Nevertheless, the typical drug, haloperidol and the atypical drug clozapine, when used at suitable doses, can achieve therapeutic effects without side effects. Indeed, both Nyberg and Farde (2000) and Remington and Kapur (2000) have emphasized that in many studies comparing other drugs to haloperidol the doses of haloperidol used are very high. In consequence, extrapyramidal side effects are seen with haloperidol, and it may appear as although other drugs afford relative protection from these side effects.

It may, therefore, be that haloperidol, and other typical drugs, which tend to have a high affinity for D_2 receptors and the atypical drugs (e.g., clozapine) that tend to have lower affinities for D_2 receptors do not differ qualitatively with respect to the mechanisms by which they achieve antipsychotic effects and extrapyramidal side effects. Both classes of drugs elicit their antipsychotic effects by binding to limbic/cortical D_2/D_3 receptors. Extrapyramidal side effects are seen if there is substantial striatal D_2 receptor occupancy (>80%). It seems that if the access of dopamine to the striatal D_2 receptors is substantially reduced then extrapyramidal side effects are seen. For some drugs additional features such as antimuscarinic (clozapine) and 5-HT₂ receptor (clozapine, risperidone), antagonistic effects may help suppress side effects. Because the higher affinity drugs can be used at lower doses, they have tended to be used in excess over that required (i.e., at higher A/K_A ratios) so that side effects are seen, but the lower affinity drugs need to be used in higher doses and so relative to the K_A for the drugs they have not been used in excess and side effects have not been seen as frequently. Indeed, even the atypical drugs may elicit side effects if used in high

doses, e.g., olanzapine, risperidone (Nyberg and Farde, 2000), at these higher doses, D_2 occupancy levels exceed the threshold for extrapyramidal side effects (Kapur et al., 1999), and the access of dopamine to the receptors is reduced. Studies on the concentrations of drugs achieved in the brains of rats lend some further support to these ideas. It was found that the higher affinity drugs such as haloperidol achieved similar brain concentrations to the lower affinity drugs, e.g., thioridazine despite the lower affinity drugs being used at a higher dose (Tsuneizumi et al., 1992; Baldessarini et al., 1993). This apparent concentration of haloperidol in the brain will increase the A/K_A ratio for a given dose of drug and render the occurrence of side effects more likely. The clinical improvement without side effects seen with lower doses of haloperidol (see above) supports this argument.

B. Kinetics of the Interactions of Dopamine and Antipsychotic Drugs at Dopamine Receptors

Kinetic considerations are also important for the actions of the antipsychotic drugs at synapses (Strange, 1997). The level of dopamine at the synapse is not fixed, and there will be changes according to the activity of the synapse and the behavior of the individual. This is rather different from the situation in the imaging experiments where presumably there are no major fluctuations in dopamine during the determinations of tracer occupancy if the subject is still. To understand how the changes in dopamine may alter the effects of the antipsychotic drugs, let us, therefore, consider a synapse where there is a set concentration of an antipsychotic drug and the level of dopamine rises. As the dopamine level increases, there will be a tendency for dopamine to bind more to the receptors and for there to be a corresponding dissociation of the antipsychotic drug to progress to a new equilibrium. Equilibrium is unlikely to be achieved but the kinetics of these processes will be dependent on the properties of the antipsychotic drug. Drugs with low values of K_d will have low dissociation rates, and drugs with high values of K_d will have faster dissociation rates. This is a consequence of the interrelationship between K_d and the ratio of dissociation and association rate constants. The association rate constants for different drugs will be similar, because this process will largely be dependent on diffusion of the drug to the receptors, so that as K_d changes so will the dissociation rate. For drugs with higher dissociation rate constants, the drug will dissociate from the receptors more quickly and may keep pace with the changes in dopamine. For drugs with low dissociation rate constants, the drug may not dissociate quickly enough to keep pace with the changing dopamine. It has been suggested that, for drugs such as clozapine and quetiapine, which have low affinities for the D_2 receptor, the dissociation rate will be fast and this will mean that dopamine will not be fully prevented from access to the D_2 receptors. For the higher affinity drugs (e.g., haloper-

idol), a fuller blockade is achieved (Kapur and Seeman, 2001), because this drug will not dissociate rapidly from the receptors. This may provide an additional safety factor in the use of the lower affinity drugs limiting their propensity for extrapyramidal side effects. It cannot, however, apply to risperidone, because this drug has an affinity for the D_2 receptor comparable to haloperidol.

The actual level of dopamine that is present in the synapse could also play a part in these effects. The synaptic level of dopamine in the striatum is higher than in the cerebral cortex (see *Section II.D.*). This will mean that the net rate of association of dopamine with the receptors in the striatum may be higher than in the cortex. As the dopamine level rises in the synapse, then the antipsychotic drug may dissociate, and the dissociation of the antipsychotic drug is likely to be the process that limits access of dopamine to the receptors for the higher affinity drugs. If, however, there is significant dissociation of drug, as may be the case for the lower affinity drugs, then depending on the actual levels of dopamine present, this may lead to differences in net rate of association of dopamine in the two tissues. Also, if the dopamine level is higher in the striatum, then this will mean that the equilibrium will lie more toward dissociation of the drug, although equilibrium is unlikely to be attained. These factors may provide for some apparent selectivity of drug action in favor of the cortex so that striatal effects of the drugs that do dissociate (lower affinity drugs) may be reduced.

C. Differential Effects of Antipsychotic Drugs in Striatal and Cortical Brain Regions

The concepts discussed in *Sections III.A.* and *III.B.* do, however, raise another issue. It seems that a typical drug such as haloperidol can be used at lower doses to achieve antipsychotic effects without extrapyramidal side effects. At these lower doses, the occupancy of striatal D_2 receptors is 50 to 65%. The occupancy of cortical/limbic D_2 receptors under these conditions is unknown, but if it is similar then this implies that this level of occupancy is sufficient to achieve a therapeutic effect. Given that 50 to 65% occupancy of striatal receptors does not produce side effects, it is difficult to see how 50 to 65% occupancy of cortical receptors could produce therapeutic effects unless dopamine mechanisms differ in the two regions. There is much evidence in favor of different dopamine mechanisms in the cerebral cortex, compared with the striatum. For example, as discussed previously, dopamine levels may be different in the two regions, and this could affect antipsychotic drug occupancies (see above). In addition, dopamine neurones in the cerebral cortex seem to behave differently, compared with those in the striatum (Lidow et al., 1998). It has been suggested that cortical dopamine systems are specialized for transmission over a wider area, compared with striatal neurones (Garris and Wightman, 1994; Jones et al., 1998). Also D_2 -like receptors in the cerebral

cortex are differentially regulated by antipsychotic drugs (Janowsky et al., 1992), compared with striatal receptors as are the neurones themselves (Robertson et al., 1994; Grace et al., 1997; Youngren et al., 1999). These mechanistic differences would then give rise to an apparent selectivity of drug action between the two brain regions. Apparent selectivity of antipsychotic drug action may be increased for kinetic reasons as discussed under *Section III.B*.

An indication of differences in the dopamine systems in the cortex and the striatum has come from studies where the occupancies of cortical and striatal dopamine receptors by different drugs have been examined. These studies have shown that, for the atypical drugs clozapine, olanzapine, quetiapine, and sertindole, occupancies were higher in cortical regions than in striatal regions (Pilowsky et al., 1997; Meltzer et al., 1999; Bigliani et al., 2000; Stephenson et al., 2000). Therefore striatal occupancy data may underestimate cortical occupancy for these drugs. Cortical occupancy data for other (typical) drugs are for doses where striatal occupancy is high, so it is difficult to determine the relative occupancies for typical drugs although a trend to greater occupancy in cortical regions can be seen (Bigliani et al., 1999). It should, however, be noted that a study of one patient has failed to replicate these differential occupancies with clozapine (Farde et al., 1997). More work needs to be done here to understand the basis of these differential occupancies, but they could be related to the differences in cortical and striatal dopamine function outlined above. It should also be noted that such occupancy data do not directly reflect the behavior of the drugs as antipsychotics; when used against psychotic symptoms, they are presumably counteracting the actions of dopamine, whereas in the imaging experiments only receptor occupancy is assessed.

In this discussion, it should not be forgotten that, in the use of antipsychotic drugs for therapy of schizophrenia, the drugs are used chronically. The therapeutic antipsychotic effects occur only after treatment for several weeks. Therefore, a discussion of differences between typical and atypical drugs must take in to account that the binding of the drug to the receptors is only the first step in a longer chain of events (see *Section IV*).

D. Occupancy of Dopamine Receptors by Clozapine: Use of Different Tracer Radioligands

There has been much discussion about the occupancy of the D_2 receptors in the striatum achieved by the atypical drug clozapine, which has been shown in many studies (see above) to be lower than that achieved by other drugs, including typical antipsychotics (e.g., haloperidol). The occupancy achieved by clozapine is particularly low when determined using methylspiperone-related tracers, but, with [^{11}C]raclopride, there is still a difference between the occupancies reported for clozapine and typical drugs. Seeman and Tallerico (1998,

1999) have suggested that occupancy data for clozapine (and quetiapine that also exhibits lower occupancies) are underestimates, and the true occupancy figure is 75 to 80%. In one hypothesis, they suggest that the underestimation results from displacement of bound clozapine or quetiapine by nontracer concentrations of PET ligand used in some studies. They report ligand binding data with 310 nM [^3H]clozapine at D_2 receptors, where 0.1 nM raclopride can displace ~50% of the bound [^3H]clozapine in 5 min. Displacement by such low concentrations of raclopride is not consistent with its dissociation constant (Table 1). Also, it is difficult to see how any specific binding of the radioligand can be detected at such a high concentration of radioligand, where the nonspecific binding must overwhelm the specific binding, so that further experimentation is required.

The different occupancies obtained with the two tracers (methylspiperone and raclopride) most likely reflect methodological differences. The methylspiperone-related tracers never reach equilibrium with the receptors during the PET experiment (Sedvall et al., 1986) owing to their slow kinetics (see earlier), whereas [^{11}C]raclopride does approach equilibrium, so, the two kinds of study are very different. In these studies, the patient has been on the drug for some time, so that the drug is likely to be at equilibrium with the receptors. The PET tracer is then given and allowed a certain time to bind to receptors, and at the same time the drug on the receptors will dissociate. In the case of [^{11}C]raclopride, the tracer is left until a new equilibrium is reached and then haloperidol is seen to occupy ~80% of the receptors (in early studies) and clozapine ~60% (Nordstrom et al., 1995). In the use of methylspiperone tracers, the experiment measures the rate of tracer binding and the reduction of this by the drug. In one study, haloperidol attenuated this by 40%, whereas clozapine had no effect (Karbe et al., 1991), thus indicating that the use of this protocol is much less sensitive to the effects of the drug. This lower sensitivity is probably a reflection of technical differences in the procedures used as the two tracers behave very differently.

The differential effects of haloperidol and clozapine in these studies are likely to be due in part to the use of the drugs so that different A/K_A ratios are achieved (see previous data). When lower doses of haloperidol are used in studies with [^{11}C]raclopride, the occupancies achieved with the two drugs are more similar (~60–65%). There does, however, seem to be a real difference in the behavior of clozapine, compared with other antipsychotic drugs in their abilities to occupy D_2 receptors in vivo. Attempts have been made by two groups to perform saturation analyses of the binding of clozapine and other drugs at D_2 receptors using PET studies in living human brain (Nordstrom et al., 1995; Kapur et al., 1999). Although these studies are difficult to perform and interpret, it seems that clozapine is able to occupy only ~60% of the receptors even at high doses, whereas the other

drugs tested are able to occupy all of the receptors at high doses. The explanation for this behavior is unclear at present, because in the *in vitro* studies clozapine behaves as a fully competitive ligand at the D_2 receptor. A further complication has recently been reported for clozapine and quetiapine in that these drugs have been found to show higher occupancies when patients are scanned soon after taking the drug, but that the apparent occupancy declines rapidly as the drug is cleared from the body (Kapur and Seeman, 2000).

E. Summary of the Differences Between Typical and Atypical Antipsychotic Drugs

It seems that we are getting nearer to understanding the propensity of different drugs to produce extrapyramidal side effects and the differences between typical and atypical antipsychotic drugs in producing such side effects. One key observation that has been made is that the typical drug haloperidol (used at low dose) and the atypical drug clozapine can be used to achieve improvement of clinical symptoms in a schizophrenic patient with minimal production of extrapyramidal side effects. In the case of haloperidol, this seems to be because the drug does not occupy all of the striatal D_2 receptors, therefore dopamine can still access these, and striatal motor function is not impaired. For clozapine, striatal D_2 receptor occupancy is also low, and there is additional kinetic protection allowing access of dopamine to the striatal D_2 receptors more readily. If the dose of drug is increased then for haloperidol, this will lead to extrapyramidal side effects because access of dopamine is prevented. For clozapine, there may be relative protection even when the dose used is higher, although it has been found that for other atypical drugs (e.g., olanzapine, risperidone) that extrapyramidal side effects can be seen if a higher dose is used.

IV. Antagonism or Inverse Agonism in the Mechanism of Antipsychotic Drugs

It is widely assumed that the antipsychotic drugs act to antagonize the actions of dopamine at synapses, in particular at the D_2 -like receptors. Recently, it has become apparent that the antipsychotic drugs are in fact inverse agonists, not antagonists when assayed at D_1 , D_2 , D_3 , and D_5 receptors expressed in recombinant systems (Nilsson and Eriksson, 1993; Charpentier et al., 1996; Griffon et al., 1996; Hall and Strange, 1997; Kozell and Neve, 1997; Malmberg et al., 1998; Wilson et al., 2001). Thus, the drugs exert effects opposite to those of dopamine. The actions at D_2 and D_3 dopamine receptors are particularly interesting because of their importance as potential sites of action of the drugs. All of the antipsychotic drugs tested exhibit inverse agonism, and this is seen independently of the type of antipsychotic drug (typical, atypical) and the chemical class. Generally, the different antipsychotic drugs exhibit similar degrees of

inverse agonism, but there is some indication in one study that different antipsychotics possess different extents of negative efficacy (Kozell and Neve, 1997).

There is then the question of whether this property of inverse agonism demonstrated in a recombinant system has any relevance to normal *in vivo* systems. Inverse agonism will be relevant to the acute effects of the antipsychotics only if there is basal (agonist-independent) activation of the receptors *in vivo*. If there is no basal activation, then an inverse agonist will be indistinguishable from a neutral antagonist in acute tests of dopamine action. The level of basal activation of dopamine receptors is unclear and difficult to measure *in vivo* owing to the presence of endogenous dopamine, but there is some indication of basal activation for the D_2 receptor in the striatum. In rats, where dopamine has been extensively depleted by 6-hydroxydopamine lesioning, effects of the antipsychotics haloperidol and clozapine have been reported (Fibiger and Robertson, 1992).

In the therapy of schizophrenia, however, the antipsychotic drugs are used chronically and it seems that that this chronic treatment is necessary to treat the positive symptoms of the disorder. The requirement for chronic treatment suggests that there is some kind of adaptive process occurring and most likely this is a change in the sensitivity of certain synapses in the brain (Strange, 1992). It is a common observation that the treatment of experimental animals with the antipsychotic drugs leads to an up-regulation of the D_2 -like receptors in the brain and this requires chronic treatment with the drug (reviewed in Sibley and Neve, 1997). It seems reasonable to suggest, therefore, that the up-regulation of D_2 -like receptors is involved in the change in synaptic efficacy that leads to the diminution of the positive symptoms. According to this theory, the up-regulation of D_2 -like receptors is central to the ability of the drugs to alter the sensitivity of dopamine synapses and hence achieve an antipsychotic effect.

This effect on dopamine receptor number has been assumed to be due to the blockade of the actions of dopamine. Indeed, prolonged treatment of experimental animals with dopamine agonists often induces down-regulation of D_2 -like receptors (Sibley and Neve, 1997), so blockade of the actions of dopamine may induce up-regulation. An alternative idea would, however, be that these effects on receptor number are due to the inverse agonist nature of the drugs. Agonists induce down-regulation and so inverse agonists may induce up-regulation. In some studies, it has been found that antipsychotic drugs induce increases in the numbers of D_2 receptors in recombinant cells expressing the receptor (reviewed in Sibley and Neve, 1997). In these experiments, there can be no competition between drug and dopamine, so the effects must be drug-related. Therefore, the ability to up-regulate receptors may be an intrinsic property of the drugs and due to their inverse agonist nature. If so, the property of inverse agonism

would be an integral part of an effective antipsychotic. The way to test this would be to identify a drug that is a neutral antagonist at D_2 -like receptors and then test this as an antipsychotic. The aminotetralin UH-232 has been shown to be a neutral antagonist (Hall and Strange, 1997) or a weak partial agonist (Coldwell et al., 1999) in studies on recombinant D_2 receptors. At the D_3 receptor, this compound behaves as a weak partial agonist (Griffon et al., 1995; Coldwell et al., 1999). Therefore, this compound differs from the conventionally used antipsychotic drugs in having a more neutral efficacy pattern taken overall. This compound has recently been tested as an antipsychotic drug and found to be devoid of antipsychotic activity (Lahti et al., 1998). This observation supports the idea that inverse agonism is central to antipsychotic action. This is a very speculative hypothesis and at present we cannot rule out the possibility that the up-regulation of receptors that may be linked to the inverse agonist property of the drugs may be associated with the side effects seen with the drugs.

V. Pharmacogenetic Studies of the Effects of Antipsychotic Drugs

There are differences in the response of different individuals to different antipsychotic drugs. For example, ~20% of patients have little response of their positive symptoms to haloperidol, and similar differences in response are seen for other antipsychotic drugs, although not necessarily in the same individuals. Some patients who do not respond to typical antipsychotic drugs will respond to clozapine, so there is much interest in clozapine in this regard. These differences in responsiveness may have a genetic basis. There is currently much interest in exploiting the explosion of information that is becoming available from the Human Genome Project to understand these differences in the actions of drugs in the human population (see for example, Roses, 2000). There are many possible reasons for these differences in drug susceptibility, including differences in target sites, differences in response systems, and differences in drug metabolizing enzymes. In this section, I shall consider some examples of genetic variants that could account for differences in antipsychotic response.

One possible source of genetic variation could be at the level of the receptors that are the targets for the antipsychotic drugs. Several variants of D_2 , D_3 , and D_4 receptors have been identified that are polymorphic in the human population and that could therefore contribute to differences in antipsychotic response (see for example, Seeman and Van Tol, 1994; Neve and Neve, 1997). It should be noted that variants can exist that result in changes in the amino acid sequence, and variants can exist that result only in a change in the DNA sequence. The former class of variants has the potential to change the affinity or response to an antipsychotic drug, whereas the latter class of variants will either be silent

or could change the expression of the receptor gene and so might affect responsiveness. There are many variants in the D_2 -like receptors that result in changes to the DNA sequence alone and a few variants that result in changes in the amino acid sequence of the receptor. I shall consider some of these latter variants that have been characterized and for a full list of the variants, see the references cited above.

For the D_2 receptor, three polymorphic variants have been analyzed: V96A, P310S, and S311C. V96A is very rare, but the other variants are found to significant extents in some populations. These variants have been examined for their ability to bind ligands and couple to signaling systems (Cravchik et al., 1996, 1999) and whereas the V96A variant shows properties similar to those of the native receptor, the P310S and S311C variants exhibit some changes. Inhibition of adenylyl cyclase by dopamine is impaired for these variants, and this is not a result of altered binding of dopamine to the receptor. These mutations are in the third intracellular loop of the receptor that is important for coupling to the G proteins. There do not appear to be changes in the affinity of the receptor variants to couple to G proteins, so the mutations may result in an impaired ability to activate G proteins. There are also some differences in the binding of antipsychotic drugs to the receptor variants and in the abilities of the drugs to inhibit the functional effects of dopamine at the receptor (Cravchik et al., 1999).

For the D_4 receptor, there was much excitement when a set of polymorphic variants of the receptor were described with different insertions in the third intracellular loop and that were polymorphic in the human population (Van Tol et al., 1992). The insertions contain repeats of 16 amino acids, and between 2 and 10 repeats can be found (with the exception of the 9-repeat version). The most common is $D_{4.4}$ being found in ~60% of the population, but $D_{4.2}$ and $D_{4.7}$ are also found to lesser extents. The variants have been well characterized and although there are minor differences in pharmacological properties, there does not appear to be any major difference in the binding of drugs, such as clozapine, in interaction with G proteins or in signaling to inhibition of adenylyl cyclase (Asghari et al., 1994, Kazmi et al., 2000). Pharmacogenetic studies have been performed to examine linkage of the variants to differences in response to clozapine in different patients, but no such linkage was found (Shaikh et al., 1993). It is quite surprising and disappointing that these polymorphic variants of the D_4 receptor do not appear to have any major functional consequence.

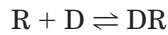
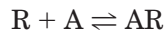
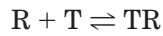
There has been much interest recently in searching for genetic markers for apparent differences in drug response in different individuals. Several polymorphisms in neurotransmitter-related genes have been identified that may predict response to clozapine, and these studies have been reviewed (Arranz and Kerwin, 2000). In a recent study, a combination of polymor-

phisms was highlighted that, if present, could predict ~80% of the response to clozapine (Arranz et al., 2000). The polymorphisms were in the serotonin 5-HT_{2A} receptor (T102C, H452Y), in the serotonin 5-HT_{2C} receptor (330GT/244CT, C23S), in the serotonin transporter promoter, and in the histamine H2 receptor (1018 GA). This is a landmark study in that it provides the first example of a genetic test for drug response and is likely to presage many further studies of this kind. It must be said, however, that it is difficult to see mechanistically how a set of six polymorphisms could influence drug response, especially when in the case discussed above, clozapine does not interact strongly with some of the targets (e.g., histamine H2 receptor).

VI. Appendix

A. Three-Way Competition Between Antipsychotic Drug, Tracer Radioligand, and Dopamine in Relation to In Vivo Imaging Studies

In an imaging experiment, the occupancy of cerebral dopamine receptors (R) by an antipsychotic drug (A) is determined by competition against a tracer radioligand (T), and there is dopamine (D) present in the vicinity of the receptors. If we assume that the system is at equilibrium, then the following equations apply.



Each of these equilibria is governed by a dissociation constant (K_T , K_A , and K_D , respectively).

It is then possible to determine an expression for the fractional occupancy of the receptors by the tracer, assuming that there is full competition for the receptor binding sites by the three ligands.

$$F = \frac{T}{T + K_T \left(1 + \frac{A}{K_A} + \frac{D}{K_D} \right)}$$

In the absence of the drug, the fractional occupancy of receptors by tracer is given by the equation

$$F = \frac{T}{T + K_T \left(1 + \frac{D}{K_D} \right)}$$

These equations can be used to determine an expression for the concentration of antipsychotic that gives 50% inhibition of tracer binding.

$$A_{50} = \frac{K_A}{K_T} \left(T + K_T \left(1 + \frac{D}{K_D} \right) \right)$$

This shows that the concentration of dopamine relative to K_D will raise the concentration of the antipsychotic needed to inhibit tracer binding, that is the value of D/K_D may affect the percent occupancy seen by an antipsychotic drug. Specifically, there will be greater occupancy in brain regions where dopamine is lower than in regions where dopamine is higher, although to have an appreciable effect on A_{50} , D/K_D will have to be greater than one. An expression for the concentrations of antipsychotic drug (A'_{50} , A''_{50}) that will give 50% inhibition of tracer binding for two different concentrations of dopamine (D' , D'') is as follows.

$$\frac{A'_{50}}{A''_{50}} = \frac{T + K_T \left(1 + \frac{D'}{K_D} \right)}{T + K_T \left(1 + \frac{D''}{K_D} \right)}$$

If the tracer is present at very low concentrations, then this reduces to

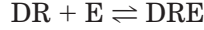
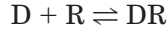
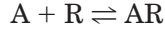
$$\frac{A'_{50}}{A''_{50}} = \frac{\left(1 + \frac{D'}{K_D} \right)}{\left(1 + \frac{D''}{K_D} \right)}$$

Whether or not there is a difference in A'_{50} and A''_{50} will depend on the values for D/K_D in the two regions. For example, in the striatum and presumably the cerebral cortex, the values for D/K_D extrasynaptically are low (~0.05, see text) and so will not affect the A_{50} values. D/K_D values synaptically are ~1 in the striatum, so that the concentration of antipsychotic required to inhibit tracer binding by 50% will be raised by a factor of ~2. In the cerebral cortex, the extrasynaptic dopamine levels are 5- to 10-fold lower, and if this is a reflection of lower synaptic dopamine levels, then in this brain region D/K_D will be lower and there will be little effect of synaptic dopamine on the A_{50} value. This may contribute to the higher receptor occupancies seen with some drugs in the cerebral cortex (see text).

B. Effect of Synaptic Dopamine Concentration on Inhibition of Functional Response by Antipsychotic Drugs

Here, we are considering the effects of the local concentration of dopamine (D) on the inhibition of responses to dopamine by antipsychotic drugs (A). An appropriate way to represent the effects of dopamine is to use the Operational model of Black and Leff (1983), but to include the effects of a competitive inhibitor, the antipsychotic drug. In this model, the interaction of the agonist/receptor complex (DR) with an effector (E) occurs with a dissociation constant (K_E). R_0 denotes the number of receptors.

The following equilibria apply:



K_A and K_D apply as in the previous section.

It can then be shown (Black and Leff, 1983) that the response in the system as a function of the maximal system response, in the absence of antipsychotic is given by

$$\frac{RESPONSE}{RESPONSE_{\max}} = \frac{Ro \cdot D}{K_D \cdot K_E + (Ro + K_E)D}$$

and the response in the system in the presence of antipsychotic is given by

$$\frac{RESPONSE_A}{RESPONSE_{\max}} = \frac{Ro \cdot D}{K'_D \cdot K_E + (Ro + K_E)D}$$

where

$$K'_D = K_D(1 + A/K_A)$$

The concentration of dopamine giving a half-maximal response (D_{50}) is then given by

$$D_{50} = \frac{K_D}{1 + \frac{Ro}{K_E}}$$

in the absence of antipsychotic and by

$$D_{50,A} = \frac{K'_D}{1 + \frac{Ro}{K_E}}$$

in the presence of antipsychotic.

Black and Leff (1983) show that if Ro/K_E is low then $D_{50} = K_D$, but if Ro/K_E is high then $D_{50} \ll K_D$, this latter condition corresponding to a receptor reserve or amplification in the system. Hence, a high value of Ro/K_E represents operationally the condition of receptor reserve or amplification in the system.

Based on the above equations, the effect of the drug will be to increase the concentration of dopamine required to give a half-maximal response, i.e.,

$$\frac{D_{50}}{D_{50,A}} = \frac{K_D}{K'_D}$$

Using the equation for the response in the system as derived by Black and Leff (1983) and given earlier, for a system receiving a set level of dopamine (D), the response in the presence of the drug relative to the response in the absence of drug is given by

$$\frac{RESPONSE_A}{RESPONSE} = \frac{K_D \cdot K_E + (Ro + K_E)D}{K'_D \cdot K_E + (Ro + K_E)D}$$

which may be rewritten as

$$\frac{RESPONSE_A}{RESPONSE} = \frac{\frac{K_D}{D} + \left(1 + \frac{Ro}{K_E}\right)}{\frac{K'_D}{D} + \left(1 + \frac{Ro}{K_E}\right)}$$

There will be no effect of the drug on the maximal response to dopamine if Ro/K_E is high, that is if there is a receptor reserve or amplification in the system, or if the level of dopamine is very high, that is K_D/D is low. If the dopamine occupancy is low, then $K_D/D > (1 + Ro/K_E)$ and

$$\frac{RESPONSE_A}{RESPONSE} = \frac{K_D}{K'_D}$$

$$\frac{RESPONSE_A}{RESPONSE} = \frac{K_A}{A + K_A}$$

if $A > K_A$ then this approximates to K_A/A , so the effect of the drug depends on its concentration relative to K_A .

Recent studies with PET scans have shown that there is a clinical improvement in patients when there is 50 to 65% occupancy of the receptors by the antipsychotic drug (Nyberg et al., 1995; Kapur et al., 2000). It has also been shown that, during a psychotic episode, there is an approximately 2-fold increase in the potential for dopamine release (Laruelle and Abi-Dargham, 1999). If we use the formula given earlier,

$$\frac{RESPONSE}{RESPONSE_{\max}} = \frac{Ro \cdot D}{K_D \cdot K_E + (Ro + K_E)D}$$

and substitute in this an increase in dopamine of 2-fold and an increase in apparent K_D of 2-fold as would be given by 50% occupancy by the antipsychotic, then the response is restored to the normal level. This is an intriguing result, but as pointed out in the text, this analysis does not take account of the need for long-term treatment with the drugs to achieve therapeutic effects or the possible differences in dopamine receptor occupancies in different brain regions.

C. Effect of Synaptic Dopamine Release on Binding of Radiotracer to Brain Dopamine Receptors in Vivo

Using the equation under Section VI.A., expressions for the fractional occupancy of receptors by radiotracer (T) under baseline conditions (F_o) and after amphetamine treatment (F_a) may be derived. The analysis assumes that all of the D_2 receptors are accessible to dopamine and radiotracer, and that the interaction is fully competitive and at equilibrium. Do and Da are the concentrations of dopamine before and after amphetamine treatment so that $Da > Do$.

$$F_o = \frac{T}{T + K_T \left(1 + \frac{Do}{K_D}\right)} \quad F_a = \frac{T}{T + K_T \left(1 + \frac{Da}{K_D}\right)}$$

TABLE 4

Effects of amphetamine on dopamine levels and radiotracer binding in the striatum of nonhuman primates

	Amphetamine	Dopamine	Radiotracer Binding
	mg/kg	fold increase over basal	% reduction
Breier et al., 1997	0.2	4.6	10.5
	0.4	13.7	21.3
Laruelle et al., 1997b	0.3	7.3	20
	0.5	10.3	28
	1.0	15.2	38

Data are taken from the studies of Breier et al. (1997) and Laruelle et al. (1997b).

under tracer conditions $T \ll K_T$ and we can define Da as nDo to reduce the equations above to

$$F_o = \frac{T}{K_T \left(1 + \frac{Do}{K_D}\right)} \quad F_a = \frac{T}{K_T \left(1 + \frac{nDo}{K_D}\right)}$$

hence,

$$\frac{F_a}{F_o} = \frac{\left(1 + \frac{Do}{K_D}\right)}{\left(1 + \frac{nDo}{K_D}\right)}$$

This equation may be used to determine values of Do/K_D from the data in Table 4 for the effects of amphetamine. In this table, values of the fold increase in dopamine over basal are given (n in the previous equation) and the percent reduction in tracer binding are given ($100 F_a/F_o$ in the previous equation). Similar calculations can be made for the effects of dopamine depletion by α MPT or reserpine.

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REFERENCES

- Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM and Laruelle M (2000) Increased baseline occupancy of D-2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci USA* **97**:8104–8109.
- Arnt J and Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the literature. *Neuropsychopharmacology* **18**:63–101.
- Arranz MJ and Kerwin RW (2000) Neurotransmitter related genes and antipsychotic response: Pharmacogenetics meets psychiatric treatment. *Ann Med* **32**:128–133.
- Arranz MJ, Munro J, Birkett J, Bolonna A, Mancama D, Sodhi M, Lesch KP, Meyer JFW, Sham P, Collier DA, Murray RM and Kerwin RW (2000) Pharmacogenetic prediction of clozapine response. *Lancet* **355**:1615–1616.
- Asghari V, Schoots O, Van Kats S, Ohara K, Jovanovic V, Guan H, Bunzow JR, Petronis A and Van Tol HHM (1994) Dopamine D_4 receptor repeat: Analysis of different native and mutant forms of the human and rat genes. *Mol Pharmacol* **46**:364–373.
- Baldessarini RJ, Centorrino F, Flood JG, Volpicelli SA, Huston-Lyons D and Cohen BM (1993) Tissue concentrations of clozapine and its metabolites in the rat. *Neuropsychopharmacology* **9**:117–124.
- Bigliani V, Mulligan RS, Acton PD, Ohlsen RI, Pike VW, Ell PJ, Gacinovic S, Kerwin RW and Pilowsky L (2000) Striatal and temporal cortical D_2/D_3 receptor occupancies by olanzapine and sertindole in vivo: A [123 I]epidepride single photon emission tomography (SPET) study. *Psychopharmacology* **150**:132–140.
- Bigliani V, Mulligan RS, Acton PD, Visivikis D, Ell PJ, Stephenson C, Kerwin RW and Pilowsky LS (1999) In vivo occupancy of striatal and temporal cortical D_2/D_3 receptors by typical antipsychotic drugs. *Br J Psychiatry* **175**:231–238.
- Black JW and Leff P (1983) Operational models of pharmacological agonism. *Proc R Soc B* **220**:141–162.
- Breier A, Su TP, Saunders TP, Carson RE, Kolachana BS, de Bartolomeir A, Weinberger DR, Weisenfeld N, Malhotra AK, Eckelman WC and Pickar D (1997)

- Schizophrenia is associated with elevated amphetamine induced synaptic dopamine concentrations: Evidence from a novel positron emission tomography method. *Proc Natl Acad Sci USA* **94**:2569–2574.
- Bristow LJ, Kramer MS, Kulagowski J, Patel S, Regan CI and Seabrook GR (1997) Schizophrenia and L 745870 a novel dopamine D_4 receptor antagonist. *Trends Pharmacol Sci* **18**:186.
- Burgisser E, Hancock AA, Lefkowitz RJ and De Lean A (1981) Anomalous equilibrium binding properties of high affinity radioligands. *Mol Pharmacol* **19**:205–216.
- Chang K, Jakobs S and Cuatrecasas P (1975) Quantitative aspects of hormone receptor interaction of high affinity. Effect of receptor concentration and measurement of affinity constants of labelled and unlabelled hormones. *Biochim Biophys Acta* **406**:294–303.
- Charpentier S, Jarvie KR, Severynse DM, Caron MG and Tiberi M (1996) Silencing of the constitutive activity of the dopamine D_{1B} receptor-reciprocal mutations between $D1$ receptor subtypes delineate residues underlying activation properties. *J Biol Chem* **271**:28071–28076.
- Coldwell MC, Boyfield I, Brown AM, Stemp G and Middlemiss DN (1999) Pharmacological characterisation of extracellular acidification rate response in human D_{2long} , D_3 and $D_{4.4}$ receptors expressed in CHO cells. *Br J Pharmacol* **127**:1135–1144.
- Cravchik A, Sibley DR and Gejman PV (1996) Functional analysis of the human D_2 dopamine receptor missense variants. *J Biol Chem* **271**:26013–26017.
- Cravchik A, Sibley DR and Gejman PV (1999) Analysis of neuroleptic binding affinities and potencies for the different human D_2 dopamine missense variants. *Pharmacogenetics* **9**:17–23.
- Creese I, Burt DR and Snyder SH (1976) Dopamine receptors and average clinical doses. *Science (Wash DC)* **194**:546.
- Farde L, Wiesel FA, Halldin C, Sedvall G and Nilsson L (1989) Dopamine receptor occupancy and plasma haloperidol levels. *Arch Gen Psychiatry* **46**:483–484.
- Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C and Sedvall G (1992) Positron emission tomography analysis of central D_2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. *Arch Gen Psychiatry* **49**:538–544.
- Farde L, Suhara T, Nyberg S, Karlsson P, Nahshima Y, Hietalen J and Halldin C (1997) A PET study of [11 C] FLB457 binding to extrastriatal D_2 dopamine receptors in healthy subjects and antipsychotic drug treated patients. *Psychopharmacology* **133**:396–404.
- Fibiger HC and Robertson GS (1992) Neuroleptics increase c-fos expression in the forebrain—contrasting effects of haloperidol and clozapine. *Neuroscience* **46**:315–328.
- Fisher RE, Morris ED, Alpert NM and Fischman AJ (1995) In vivo imaging of neuromodulatory synaptic transmission using PET: A review of relevant neurophysiology. *Hum Brain Mapping* **3**:24–34.
- Gardner B, Hall DA and Strange PG (1997) Agonist action at $D_{2(short)}$ dopamine receptors determined in ligand binding and functional assays. *J Neurochem* **69**:2589–2598.
- Garris PA and Wightman RM (1994) Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex and striatum, an in vivo voltammetry study. *J Neurosci* **14**:442–450.
- Gerlach J (2000) Atypical antipsychotics: An inspiring but confusing concept. *Psychopharmacology* **148**:1–2.
- Genovart N, Farde L, Halldin C and Swahn CG (1997) Effect of reserpine induced depletion of synaptic dopamine on [11 C]raclopride binding to D_2 dopamine receptors in monkey brain. *Synapse* **25**:321–325.
- Golds PR, Przyslo FR and Strange PG (1980) The binding of some antidepressant drugs to brain muscarinic acetylcholine receptors. *Br J Pharmacol* **68**:541–549.
- Grace AA, Bunney BS, Moore H and Todd CL (1997) Dopamine cell depolarisation block as a model for the therapeutic actions of antipsychotic drugs. *Trends Neurosci* **20**:31–37.
- Griffon N, Pilon C, Schwartz JC and Sokoloff P (1995) The preferential dopamine D_3 receptor ligand (+)-UH 232 is a partial agonist. *Eur J Pharmacol* **282**:R3–R4.
- Griffon N, Pilon C, Sautel F, Schwartz JC and Sokoloff P (1996) Antipsychotics with inverse agonist activity at the D_3 dopamine receptor. *J Neural Transm* **103**:1163–1175.
- Hagberg G, Gefvert O, Bergstrom M, Wieselgren IM, Lindstrom L, Wiesel FA and Langstrom B (1998) N-[C-11]methylspiperone PET, in contrast to [C-11]raclopride, fails to detect D_2 receptor occupancy by an atypical neuroleptic. *Psychiatr Res—Neuroimaging* **82**:147–160.
- Hall DA and Strange PG (1997) Evidence that antipsychotic drugs are inverse agonists at D_2 dopamine receptors. *Br J Pharmacol* **121**:731–736.
- Hoare SR and Strange PG (1996) Regulation of D_2 dopamine receptors by amiloride and amiloride analogues. *Mol Pharmacol* **50**:1295–1308.
- Hulme EC and Birdsall NJM (1992) Strategy and tactics in receptor binding studies, in *Receptor-Ligand Interactions—A Practical Approach*, pp 63–176, IRL Press, Oxford.
- Janowsky A, Neve KA, Kintzel M, Taylor B, de Paulis T and Bellkamp JK (1992) Extrastriatal dopamine D_2 receptors: Distribution, pharmacological characterisation and regionally specific regulation by clozapine. *J Pharmacol Exp Ther* **261**:1282–1290.
- Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM and Caron MG (1998) Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proc Natl Acad Sci USA* **95**:4029–4034.
- Joyce JN, Janowsky A and Neve KA (1991) Characterisation and distribution of [125 I]epidepride binding to D_2 receptors in basal ganglia and cortex of human brain. *J Pharmacol Exp Ther* **257**:1253–1263.
- Kane JM (1997) What makes an antipsychotic atypical? Should the definition be preserved. *CNS Drugs* **7**:347–348.
- Kapur S and Seeman P (2001) Fast dissociation from the dopamine D_2 receptors explains atypical antipsychotic action—A new hypothesis *Am J Psychiatry*, in press.
- Kapur S, Zipursky R, Jones C, Remington G and Houle S (2000) Relationship

- between dopamine D₂ occupancy, clinical response and side effects—A double blind PET study in first episode schizophrenia. *Am J Psychiatry* **157**:514–520.
- Kapur S, Zipursky RB and Remington G (1999) Clinical and theoretical implications of 5-HT₂ and D₂ receptor occupancy of clozapine, risperidone and olanzapine in schizophrenia. *Am J Psychiatry* **156**:286–293.
- Karbe H, Wienhard K, Hamacher K, Huber M, Herholz K, Coenen HH, Stocklin G, Lovenich A and Heiss WD (1991) Positron emission tomography with ¹⁸F methylspiperone demonstrates D₂ dopamine receptor binding differences of clozapine and haloperidol. *J Neural Transm Gen Sect* **86**:163–173.
- Kawagoe KT, Garriss PA, Wiedemann DJ and Wightman RM (1992) Regulation of transient dopamine concentration gradients in the microenvironment surrounding nerve terminals in the rat striatum. *Neuroscience* **51**:55–64.
- Kazmi MA, Snyder LA, Cypess AM, Graber SG and Sakmer TP (2000) Selective reconstitution of human D₄ dopamine receptor variants with G_{ia} subtypes. *Biochemistry* **39**:3734–3744.
- Koepp MJ, Gunn RN, Lawrence AD, Cunningham VJ, Dagher A, Jones A, Brooks DJ, Bench CJ and Grasby PM (1998) Evidence for striatal dopamine release during a video game. *Nature (Lond)* **393**:266–268.
- Kozell LB and Neve KA (1997) Constitutive activity of a chimeric D₂/D₁ dopamine receptor. *Mol Pharmacol* **52**:1137–1149.
- Lahti CA, Weiler M, Carlsson A and Tamminga CA (1998) Effects of the D-3 and autoreceptor preferring dopamine antagonist (+)-UH 232 in schizophrenia. *J Neural Trans* **105**:719–734.
- Laruelle M (2000) Imaging synaptic neurotransmission with *in vivo* binding competition techniques: A critical review. *J Cereb Blood Flow Metab* **20**:423–451.
- Laruelle M and Abi-Dargham A (1999) Dopamine as the wind of the psychotic fire, new evidence from brain imaging studies. *J Psychopharmacol* **13**:358–371.
- Laruelle M, Abi-Dargham A, Gil R, Kegeles L and Innis R (1999) Increased dopamine transmission in schizophrenia: Relationship to illness phases. *Biol Psychiatry* **46**:56–72.
- Laruelle M, D'Souza CD, Baldwin RM, Abi-Dargham A, Kanes SJ, Fingado CL, Seibyl JP, Zoghbi SS, Bowens MB, Jatlow P, Charney DS and Innis RD (1997a) Imaging D₂ receptor occupancy by endogenous dopamine in humans. *Neuropsychopharmacology* **17**:162–174.
- Laruelle M, Iyer RN, Al-Tikriti MS, Zea-Ponce Y, Malison R, Zoghbi SS, Baldwin RM, Kung HK, Charney DS, Hoffer PB, Innis RB and Bradberry CW (1997b) Microdialysis and SPECT measurements of amphetamine induced dopamine release in nonhuman primates. *Synapse* **25**:1–14.
- Lidow MS, Williams GV and Goldman-Rakic PS (1998) The cerebral cortex: A case for a common site of action of antipsychotics. *Trends Pharmacol Sci* **19**:136–140.
- Malmberg A, Jackson DM, Eriksson A and Mohell N (1993) Unique binding characteristics of antipsychotic agents interacting with human dopamine D_{2A}, D_{2B} and D₃ receptors. *Mol Pharmacol* **43**:749–754.
- Malmberg A, Jerning E and Mohell N (1996) Critical re-evaluation of spiperone and benamide binding to dopamine D₂ receptors: Evidence for identical binding sites. *Eur J Pharmacol* **303**:123–128.
- Malmberg A, Mikaelis A and Mohell N (1998) Agonist and inverse agonist activity at the dopamine D₃ receptor measured by guanosine 5'-[γ-thio]triphosphate [³⁵S] binding. *J Pharmacol Exp Ther* **285**:119–126.
- McEvoy JP, Hogarty GE and Steingard S (1991) Optimal dose of neuroleptic for schizophrenia: A controlled study of neuroleptic threshold and higher haloperidol dose. *Arch Gen Psychiatry* **48**:739–745.
- Meltzer HY, Park S and Kessler R (1999) Cognition, schizophrenia and the atypical antipsychotic drugs. *Proc Natl Acad Sci USA* **96**:13591–13593.
- Moore H, West AR and Grace AA (1999) The regulation of forebrain dopamine transmission: Relevance to the pathophysiology and psychopathology of schizophrenia. *Biol Psychiatry* **46**:40–55.
- Motulsky HJ and Mahan LC (1984) The kinetics of competitive radioligand binding predicted by the law of mass action. *Mol Pharmacol* **25**:1–9.
- Neve KA and Neve RL (1997) The dopamine receptors, in *Molecular Biology of Dopamine Receptors* (Neve KA and Neve RL eds) pp 27–76, Humana Press, Totowa, NJ.
- Nilsson CL and Eriksson F (1993) Haloperidol increases prolactin release and cAMP formation in vitro-inverse agonism at dopamine D₂ receptors. *J Neural Transm* **92**:213–220.
- Nyberg S and Farde L (2000) Non-equipotent doses partly explain differences among antipsychotics—Implications for PET studies. *Psychopharmacology* **148**:22–23.
- Nyberg S, Farde L, Halldin C, Dahl ML and Bertelson L (1995) D₂ receptor occupancy during low dose treatment with haloperidol decanoate. *Am J Psychiatry* **152**:173–178.
- Nordstrom A, Farde L, Nyberg S, Karlsson P, Halldin C and Sedvall G (1995) D₁, D₂ and 5HT₂ receptor occupancy in relation to clozapine serum concentration. A PET study of schizophrenic patients. *Am J Psychiatry* **152**:1444–1449.
- Nordstrom A, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C and Uppfeldt G (1993) Central D₂ dopamine receptor occupancy in relation to antipsychotic effect: A double blind PET study of schizophrenic patients. *Biol Psychiatry* **33**:227–235.
- Patel S, Freedman S, Chapman KL, Emms F, Fletcher AE, Knowles M, Marwood R, McAllister G, Myers J, Patel S, Curtis N, Kulagowski JJ, Leeson PD, Ridgill M, Graham M, Matheson S, Rathbone D, Watt AP, Bristow LJ, Rupniak NMJ, Baskin E, Lynch JJ and Regan CI (1997) Biological profile of L-745,870 a selective antagonist for the dopamine D₄ receptor. *J Pharmacol Exp Ther* **283**:636–647.
- Picini P, Brooks DJ, Bjorkland A, Gunn RN, Grasby PM, Rimoldi O, Brundin P, Hagell P, Rehnrota S, Widner H and Lindvall O (1999) Dopamine release from nigral transplants visualised in vivo in a Parkinson's disease patient. *Nature Neurosci* **2**:1137–1140.
- Pickar D, Su T, Weinberger DR, Coppola R, Malhotra AK, Knable MB, Lee KG, Gorey J, Bartko SJ, Breier A and Hsiao J (1996) Individual variation in D₂ dopamine receptor occupancy in clozapine treated patients. *Am J Psychiatry* **153**:1571–1578.
- Pilowsky L, Mulligan RS, Acton PD, Eil PJ, Costa DC and Kerwin RW (1997) Limbic selectivity of clozapine. *Lancet* **350**:490–491.
- Remington G and Kapur S (2000) Atypical antipsychotics: Are some more atypical than others. *Psychopharmacology* **148**:3–15.
- Robertson GS, Matsumura H and Fibiger HC (1994) Induction patterns of fos like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. *J Pharmacol Exp Ther* **271**:1058–1066.
- Roses A (2000) Pharmacogenetics and future drug development and delivery. *Lancet* **355**:1358–1361.
- Schotte A, Janssen PFM, Gommeren W, Luyten WHML, Van Gompel P, Lesage AS, De Loove K and Leysen J (1996) Risperidone compared with new and reference antipsychotic drugs: In vitro and in vivo receptor binding. *Psychopharmacology* **124**:57–73.
- Sedvall G, Farde L, Persson A and Wiesel FA (1986) Imaging of neurotransmitter receptors in the living human brain. *Arch Gen Psychiatry* **43**:995–1005.
- Seeman P (1992) Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D₂ receptors, clozapine occupies D₄ receptors. *Neuropsychopharmacology* **7**:261–284.
- Seeman P, Lee T, Chau-Wong M and Wong K (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature (Lond)* **261**:717–719.
- Seeman P and Tallerico T (1998) Antipsychotic drugs which elicit little or no Parkinsonism bind more loosely than dopamine to brain D₂ receptors, yet occupy higher levels of these receptors. *Mol Psychiatry* **3**:123–134.
- Seeman P and Tallerico T (1999) Rapid release of antipsychotic drugs from dopamine D₂ receptors: An explanation for low receptor occupancy and early clinical relapse upon withdrawal of clozapine or quetiapine. *Am J Psychiatry* **156**:876–884.
- Seeman P, Ulpian C, Wreggett KA and Wells JA (1984) Dopamine receptor parameters detected by [³H]spiperone depend on tissue concentration: Analysis and examples. *J Neurochem* **43**:221–235.
- Seeman P and Van Tol HHM (1994) Dopamine receptor pharmacology. *Trends Pharmacol Sci* **15**:264–269.
- Seeman P and Van Tol HHM (1995) Deriving the therapeutic concentrations for clozapine and haloperidol: The apparent dissociation constant of a neuroleptic at the dopamine D₂ or D₄ receptor varies with the affinity of the competing ligand. *Eur J Pharmacol* **291**:59–66.
- Shaikh S, Collier D, Kerwin RW, Pilowsky LS, Gill M, Xu W and Thornton A (1993) Dopamine D₄ receptor subtypes and response to clozapine. *Lancet* **341**:116.
- Sharp T, Zetterstrom T and Ungerstedt U (1986) An in vivo study of dopamine release and metabolism in rat brain regions using intracerebral dialysis. *J Neurochem* **47**:113–122.
- Sibley DR, Mahan LC and Creese I (1983) Dopamine receptor binding on intact cells. *Mol Pharmacol* **23**:295–302.
- Sibley DR and Neve KA (1997) The dopamine receptors, in *Molecular Biology of Dopamine Receptors* (Neve KA and Neve RL eds) pp 383–424, Humana Press, Totowa, NJ.
- Stephenson CME, Bigliani VB, Jones HM, Mulligan RS, Acton PD, Visvikis D, Eil PJ, Kerwin RW and Pilowsky LS (2000) Striatal and extra-striatal D₂/D₃ dopamine receptor occupancy by quetiapine in vivo—A [¹²³I]epidepride single photon emission tomography (SPET) scanning study. *Schizophrenia Res* **36**:247–248.
- Strange PG (1992) *Brain Biochemistry and Brain Disorders*, pp 226–257, Oxford University Press, Oxford, UK.
- Strange PG (1997) Commentary on atypical neuroleptics. *Neuropsychopharmacology* **16**:116–122.
- Tsukada H, Nishiyama S, Kakiuchi T, Ohba H, Sato K and Harada N (1999) Is synaptic dopamine concentration the exclusive factor which alters the in vivo binding of [¹¹C]raclopride? PET studies combined with microdialysis in conscious monkeys. *Brain Res* **841**:160–169.
- Tsunazumi T, Babb SM and Cohen BM (1992) Drug distribution between blood and brain as a determinant of antipsychotic drug efficacy. *Biol Psychiatry* **32**:817–824.
- Van Tol HHM, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB and Civelli O (1991) Cloning of a gene for a human D₄ dopamine receptor with high affinity for the antipsychotic clozapine. *Nature (Lond)* **350**:1–5.
- Van Tol HHM, Wu CM, Guan H, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HM and Jovanovic V (1992) Multiple dopamine D₄ receptor variants in the human population. *Nature (Lond)* **358**:149–152.
- Waddington JL and O'Callaghan E (1997) What makes an antipsychotic atypical? Conserving the definition. *CNS Drugs* **7**:341–346.
- Wells JW, Birdsall NJM and Burgen ASV (1980) Competitive binding studies with multiple sites. Effects arising from depletion of the free ligand. *Biochim Biophys Acta* **632**:464–469.
- Whetzel SZ, Shih YH, Georgic LM, Akunne HC and Pugsley TA (1997) Effects of the dopamine D₃ antagonist PD 58491 and its interaction with the dopamine D₃ agonist PD 128907 on brain dopamine synthesis in the rat. *J Neurochem* **69**:2363–2368.
- Wilson J, Lin H, Fu D, Javitch JA and Strange PG (2000) Mechanisms of inverse agonism of antipsychotic drugs at the D₂ dopamine receptor: Use of a mutant D₂ dopamine receptor that adopts the activated conformation. *J Neurochem*, in press.
- Youngren KD, Inglis FM, Pivrotto PJ, Jedema HP, Bradberry CW, Goldman Rakic P, Roth RH and Moghaddam B (1999) Clozapine preferentially increases dopamine release in the rhesus monkey prefrontal cortex compared with caudate nucleus. *Neuropsychopharmacology* **20**:403–412.
- Yung KKL, Bolam JP, Smith AD, Hersch SM, Ciliax BJ and Levey AI (1995) Immunocytochemical localisation of D₁ and D₂ dopamine receptors in the basal ganglia of the rat—Light and electron microscopy. *Neuroscience* **65**:709–730.