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All Roads to Schizophrenia Lead to Dopamine Supersensitivity and Elevated Dopamine D2$^{\text{High}}$ Receptors

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SUMMARY

Background: The dopamine D2 receptor is the common target for antipsychotics, and the antipsychotic clinical doses correlate with their affinities for this receptor. Antipsychotics quickly enter the brain to occupy 60–80% of brain D2 receptors in patients (the agonist aripiprazole occupies up to 90%), with most clinical improvement occurring within a few days. The D2 receptor can exist in a state of high-affinity (D2$^{\text{High}}$) or in a state of low-affinity for dopamine (D2Low). **Aim:** The present aim is to review why individuals with schizophrenia are generally supersensitive to dopamine-like drugs such as amphetamine or methylphenidate, and whether the D2$^{\text{High}}$ state is a common basis for dopamine supersensitivity in the animal models of schizophrenia. **Results:** All animal models of schizophrenia reveal elevations in D2$^{\text{High}}$ receptors. These models include brain lesions, sensitization by drugs (amphetamine, phencyclidine, cocaine, corticosterone), birth injury, social isolation, and gene deletions in pathways for NMDA, dopamine, GABA, acetylcholine, and norepinephrine. **Conclusions:** These multiple abnormal pathways converge to a final common pathway of dopamine supersensitivity and elevated D2$^{\text{High}}$ receptors, presumably responsible for psychotic symptoms. Although antipsychotics alleviate psychosis and reverse the elevation of D2$^{\text{High}}$ receptors, long-term antipsychotics can further enhance dopamine supersensitivity in patients. Therefore, switching from a traditional antipsychotic to an agonist antipsychotic (aripiprazole) can result in psychotic signs and symptoms. Clozapine and quetiapine do not elicit parkinsonism or tardive dyskinesia because they are released from D2 within 12 to 24 h. Traditional antipsychotics remain attached to D2 receptors for days, preventing relapse, but allowing accumulation that can lead to tardive dyskinesia. Future goals include imaging D2$^{\text{High}}$ receptors and desensitizing them in early-stage psychosis.

Multiple Causes of Schizophrenia

There are many risk factors, genetic and nongenetic, for the development of schizophrenia. Genetic risk factors are numerous, with 50 to 100 gene mutations and variations reported each year as associated with schizophrenia. Nongenetic risk factors include infection during fetal life, brain injury, and anoxia at birth, trauma in childhood, abuse of street drugs and steroids, brain lesions, psychosocial stress, isolation, smoking, and excess coffee.

It is difficult to decide whether a factor is actually causative because, in the end, the signs and symptoms are the same, regardless of the cause. They are the same whether one has had a brain injury and developed schizophrenia, or whether one has smoked too much cannabis and developed schizophrenia.

In other words, despite the many different biopsychosocial origins of schizophrenia, the clinical signs, symptoms, and natural progress of the illness are more or less similar. Such similarities suggest that there may

be a common pathway through which the various causal factors operate.

Common Biomarker for Schizophrenia? Dopamine Supersensitivity

Although many biomarkers have been suggested as indicative of schizophrenia, none have stood the test of time.

Possibly, the most consistent biological aspect of schizophrenia is that the majority of patients, regardless of the apparent cause, are behaviorally supersensitive to dopamine-like drugs such as amphetamine, methamphetamine, cocaine, apomorphine, or methylphenidate, whether or not they are taking antipsychotics [1,2].

As reviewed by Lieberman et al. [1], 74–78% of patients with schizophrenia become worse with new or intensified psychotic symptoms after being given amphetamine or methylphenidate. Psychotic symptoms can also be elicited in this way in control subjects, but only in about 25% of individuals.

In addition, the worsening of symptoms caused by the psychostimulants also occurs when patients are taking antipsychotics. Altogether, psychotogens elicit or enhance psychotic symptoms in 40% of schizophrenia patients compared to ~2% of control individuals.

Because amphetamine increases the release of dopamine from dopamine neuron terminals, the psychotic action of amphetamine could arise from enhanced release of dopamine in schizophrenia [3] or from supersensitivity of postsynaptic dopamine receptors. Furthermore, by stimulating postsynaptic dopamine receptors, apomorphine can increase thought disorder [4], Thus, the common psychotogenic actions of these dopamine-like drugs is their net stimulation of postsynaptic dopamine receptors, especially D2 receptors.

The Common Target for Antipsychotics Is the Dopamine D2 Receptor. Comparison with D3 and D4

Five dopamine receptors have been found: D1, D2, D3, D4, and D5. The D2 receptor has three main variants, D2Short, D2Long, and D2Longer [5].

Of the five different dopamine receptors, D2 is the most relevant clinically, because it is the main target for antipsychotics and for dopamine-like stimulants used to alleviate Parkinson's disease [6,7].

In fact, the action of amphetamine and its blockade by antipsychotics led Van Rossum to propose that antipsychotics selectively targeted dopamine receptors [8–11]. Before 1967, antipsychotics were known to affect metabolism of adrenaline, noradrenaline, and serotonin, but no selective action on these or any other neurotransmission systems had been shown, nor were neurotransmitter receptors then directly detectable. Van Rossum's suggestion of an isolated tissue that would selectively respond to dopamine later materialized with the advent of a radioreceptor assay, using [³H]haloperidol on homogenized tissue [12–16] and later on cloned D2 receptors [17]. For the first time, this work provided direct evidence that all antipsychotics selectively blocked dopamine receptors with clinical potencies that correlated with their affinity for a dopamine receptor in vitro [13,14]. Based on these early findings, the target for the antipsychotic drugs was named "the antipsychotic/dopamine receptor" [14], but was later renamed the dopamine D2 receptor [18,19].

Apart from D2, the other four dopamine receptors are not central to the action of antipsychotics or anti-Parkinson medication. For example, the concentrations of antipsychotics that block D1 and D5 receptors are in the micromolar range [20–23], concentrations that would be lethal if found in the plasma of patients. Moreover, there is no correlation between the clinical doses of antipsychotics and their affinities for D1 or D5 [22,23].

Similarly, the dopamine D3 and D4 receptors are not clinically relevant in the action of antipsychotics, because neither of these receptors serves as a clinically consistent target for antipsychotic drugs [24,25]. Although the affinities of antipsychotics are similar for D2 and D3 receptors [26], D3 is less sensitive to haloperidol, molindone, olanzapine, quetiapine, risperidone, and especially remoxipride.

It is only the D2 receptor that is universally occupied to a therapeutic level of 60–80% by the antipsychotics [22]. The D3 receptors are not occupied by therapeutic doses of antipsychotics [24]. This observation suggests that D3 may not be a treatment target in schizophrenia. In fact, BP8947, a partial agonist with a Ki of 0.9 nM for D3, was not effective in schizophrenia (10 mg/day for 10 days)[25].

Many antipsychotic drugs have similar potencies on D2 and D4, except clozapine, which is about 10 times more potent on D4. Compared to their potency on D2, raclopride, remoxipride, sulpiride, flupentixol, and fluphenazine are all much weaker on D4 [22,27,28].

The dissociation constant of a particular antipsychotic often differs among laboratories. One reason for this is that the final concentration of tissue is different in different laboratories [29]. Another reason is that an antipsychotic shows a higher dissociation constant when competing versus a highly fat-soluble ligand, as compared to its competition versus a more water-soluble ligand [30,31]. For example, haloperidol has a dissociation constant of 0.74 nM with [³H]raclopride, 2.7 nM with

Table 1 Antipsychotic dissociation constants, Ki, at dopamine receptors

Human clone	D1	D2	D2	D2	D3	D4	5HT2A
	nM	nM	nM	nM	nM	nM	nM
[³H]ligand used	Sch.	Raclo.	Spip.	Nem.	Raclo.	Spip.	Ket.
Kd of ligand, nM		1.9	0.065	0.068	1.6	0.086	
Amisulpride-(-)-S	–	1.8	4.6	8	3	–	–
Amoxapine	–	21	56	140	–	5.5	0.6
Aripiprazole	–	1.8	–	–	–	–	–
Bifeprunox	–	3.8	–	–	–	–	–
Butaclamol–(+)	–	0.14	0.9	2.3	–	70	–
Chlorpromazine	16.5	1.2	4.6	14	1.4	9.6	2
Clozapine	90	76	180	385	190	22	4
Clozapine-iso	120	15	60	130	21	21	2.2
[³H]Domperidone	–	0.35	–	–	1.2	–	–
Droperidol	–	0.54	2.3	4	–	2	–
Flupentixol-cis	1.8	0.38	0.7	2.8	0.7	15	–
Fluphenazine	2.6	0.55	1.2	1.9	0.17	30	3.8
Haloperidol	55	0.74	2.7	8.4	8.8	2	74
Iloperidone (HP873)	5.6	5.4	10	20	20	9.6	0.2
Loxapine	18	9.8	23	41	7.2	8	1.9
Melperone	148	152	375	564	315	720	180
Metoclopramide	–	16	–	–	–	–	–
Molindone	1558	4.9	15	33	44	3900	5200
Moperone	–	1.6	3.6	5.6	–	7	–
Nemonapride	–	0.014	0.038	0.076	–	–	–
Norclozapine	73	180	300	650	–	120	–
Olanzapine	9.2	7.4	21	46	14	15	3.4
Perlapine	–	138	450	1300	168	186	22
Perphenazine	4.5	0.27	0.47	0.9	0.23	32	–
Pimozide	–	1.4	0.95	–	–	–	–
Prochlorperazine	7.7	1.7	4	5.3	–	89	–
Quetiapine	290	140	680	1400	240	2000	135
Raclopride	–	1.6	7.1	22	2.9	2400	4400
Remoxipride	4900	67	800	900	960	2400	6600
Risperidone	42	1.09	4	19	3.5	4.4	0.2
Risperidone-9-OH	–	1.6	–	–	–	–	–
Sertindole	22	1.9	6.5	8.2	3	11	0.28
Spiperone	–	0.018	0.06	0.13	–	–	0.57
Sulpiride-S	–	9.9	8	20	10	1000	–
Thioridazine	5.8	1.1	5.2	18.5	1.9	11	1.3
Trifluperazine	2.9	1.4	3.8	6.8	0.7	39	8.8
Trifluperidol	–	0.44	–	–	–	0.9	–
Ziprasidone	9	2.7	6	11	1.5	8	3

Ki (3–14 replicates) measured at ligand concentrations of 2 × Kd of ligand [26,30]

Dashes indicate "not done."

D2, D2Long; Sch, Schering 23390; Raclo., Raclopride; Spip., spiperone; Nem., Nemonapride; Ket., Ketanserin.

[³H]spiperone, and 8.4 nM with [³H]nemonapride (from Table 1).

Using the data in Table 1, Figure 1 shows that the daily oral doses of antipsychotic drugs are related to their affinities for D2, using [³H]raclopride on the human cloned D2 [31,32]. Although the doses for chlorpromazine and thioridazine are off the correlation, these two drugs are highly bound to plasma proteins. When allowance is made for this high binding to plasma pro-teins, the dissociation constants and their unbound (or free) concentrations of the antipsychotics (in the plasma water of patients) have almost identical values [21,22].

Also, the glutamate agonist LY404,039 is included in the correlation in Figure 1. Although it is a glu-tamate agonist, this compound has an antipsychotic effect [33]. However, using the D2-selective ligand [³H]domperidone, LY404,039 has an affinity for the dopamine D2^{High} receptor with a dissociation constant of

Figure 1 The clinical doses of antipsychotic medications are related to their affinities for the dopamine D2 receptor. The antipsychotic dissociation constants at D2, obtained using [3H]raclopride, are shown on the ordinate. The glutamate agonist LY404,039 [33] has an affinity for the dopamine D2High receptor with a dissociation constant of 10 nM at D2High (using [3H]domperidone, because [3H]raclopride does not readily reveal D2High receptors)[34]. This value of 10 nM predicts a clinical dose of approximately 60–100 mg/day, in general agreement with the dose of 80 mg/day used by Patil et al. [33]. Because of the very high binding (exceeding 98%) of chlorpromazine and thioridazine to plasma proteins [21], these antipsychotics require high daily doses. However, the final concentrations of all the antipsychotics (including chlorpromazine and thioridazine) in the plasma water in treated patients are almost identical to their dissociation constants [21,23](Adapted from [23]; with permission from Scholarpedia; Reproduced from [31], with permission of Walsh Medical Media LLC.)
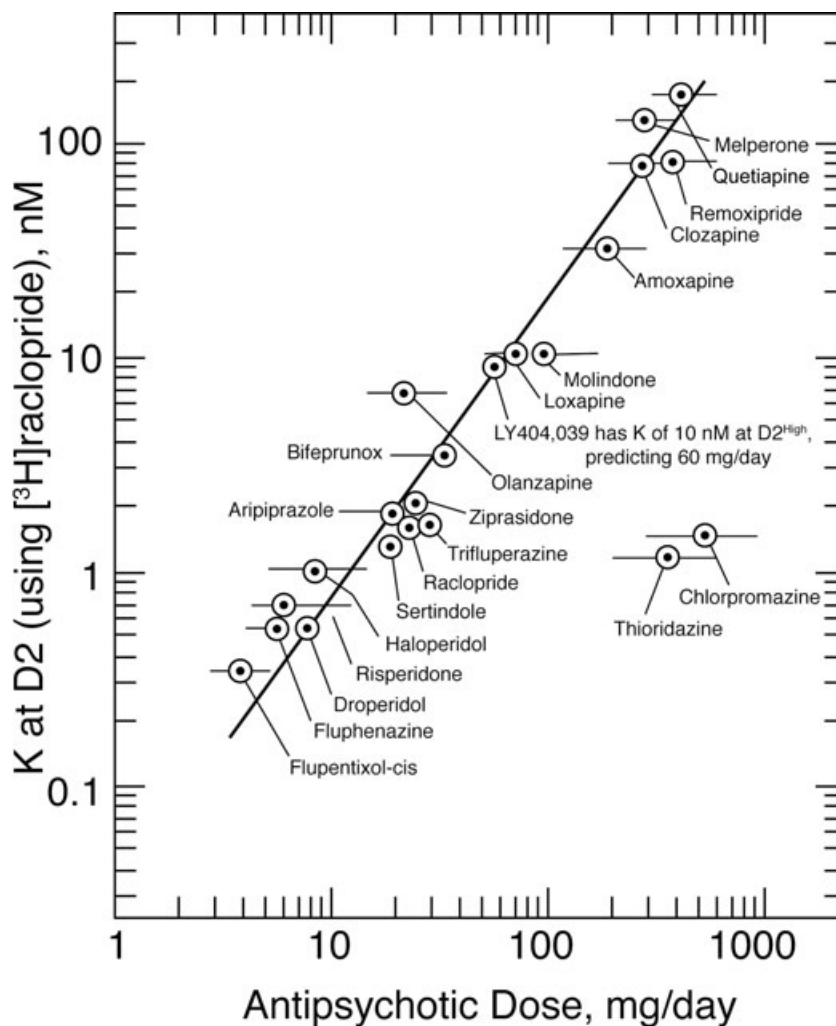
about 10 nM, as shown in Figure 2 [23,34], a concentration that would predict a clinical daily dose of approximately 60–100 mg per day for psychosis [32]; Patil et al. [33] used a daily dose of 80 mg. Therefore, because D2 is a central target for antipsychotic action and anti-Parkinson action, it is reasonable to consider whether any particular property of D2 may be related to dopamine supersensitivity and schizophrenia.

Is Dopamine Supersensitivity Related to D2 Density?

Since 1975 when the D2 receptor was discovered, it has been difficult to relate the total density of D2 receptors with behavioral dopamine supersensitivity. This is because sensitivity to a dopamine agonist increases 3-fold after denervation or after long-term antipsychotics, but the total density of D2 receptors increases by only

1.3-fold. Moreover, even though most patients with schizophrenia are supersensitive to dopamine, the density of the total population of D2 receptors is elevated by only 1.4-fold in postmortem human schizophrenia striatal tissues [35].

Apart from the modest elevations in D2 receptors in schizophrenia, a more relevant question is whether the functional state of D2, or D2High, is elevated in dopamine supersensitive animal models and in schizophrenia.

Is Dopamine Supersensitivity Related to D2High Receptors?

Dopamine has different affinities for the two states of D2. Dopamine attaches to D2High below 100 nM, and attaches to D2Low above 100 nM [36]. The D2High receptor is the functionally active state of the dopamine D2 receptor [37]. Antipsychotic drugs, however, have
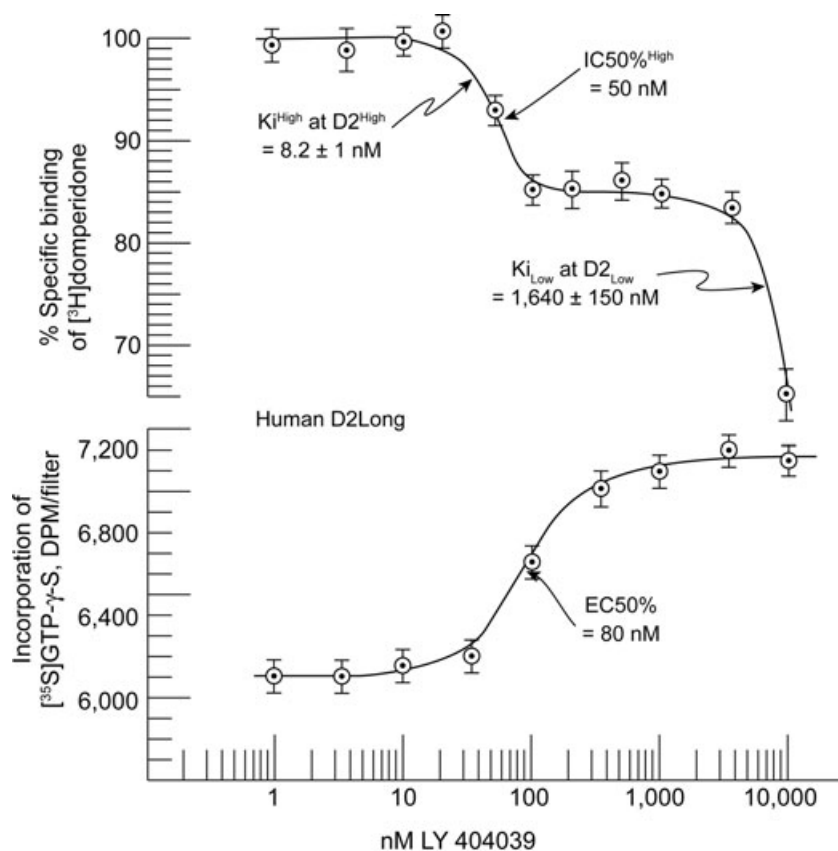
Figure 2 Top: The glutamate agonist LY404,039 inhibited the binding of 2 nM [^{3}H]domperidone on dopamine D2Long receptors (in CHO cells). The inhibition occurred in two concentration phases of LY404,039, with 15.5% inhibition for the high-affinity phase. 120 mM NaCl present. Data points are mean values (with SE; n = 3). The inhibition of 15.5% occurred at D2^{High} receptors, all of which were converted to low-affinity D2Low receptors in the presence of GN (200 μM guanylylimidodiphosphate). The dissociation constant, Ki High of LY404,039 was 8.2 ± 1 nM. Nonspecific binding was defined in the presence of 10 μM S-sulpiride. Bottom: LY404,039 stimulated the incorporation of [^{35}S]GTP-γ-S into dopamine D2Long receptors with 50% incorporation occurring at 80 ± 15 nM. The maximum amount of stimulation was 43% of that caused by 10 μM dopamine. The stimulation was blocked by 10 μM S-sulpiride. Data points are means ± SE (n = 3). (Reproduced from [34], with permission of Wiley-Liss, Inc.).

identical affinity for the high-affinity and low-affinity states of D2.

In the amphetamine-sensitized animal model of psychosis, the animal is supersensitive to dopamine-like drugs, but the density of D2 in the brain striatum is normal [36,38]. Surprisingly, however, D2^{High} receptors in the striatum were found to be markedly elevated, by 250%.

This type of elevation in D2^{High} receptors also occurred in other animal models of psychosis [36,38]. For example, mice with specific gene deletions have now been examined. A typical example is shown in Figure 3 for striata from mice with the metabotropic glutamate receptor-2 (mGluR2) gene knocked out [39]. Such mice are dopamine-supersensitive, and, while the D2 density in the striata is normal in these mice, the proportion of D2^{High} receptors increased 3- or 4-fold, as shown in Figure 3.

Additional data for many other animal models of schizophrenia are summarized in Figure 4. In general, the gene-deleted mice (gene knockout mice) showed behavioral dopamine supersensitivity and revealed elevated levels of D2^{High} receptors in the striata. Gene knockout mice with no behavioral supersensitivity did not re-

veal any elevation in the proportion of D2^{High} receptors (Figure 4, right side).

Deletions of GABA, Glutamate, and Nondopamine Genes Increase D2^{High}

Deletions of genes that are not related to the dopamine system also yield animal models of behavioral dopamine supersensitivity and at the same time reveal marked elevations in D2^{High} receptors [36,38]. These genes include those for the GABAB1 receptor, RIIβ protein kinase A, PSD95 (Post-Synaptic Density protein 95), GPRK6 (G-Protein Receptor Kinase 6), the trace amine-1 receptor, and RGS9–2 (Regulator of G protein Signaling 9–2) (Figure 4).

Many gene knockouts, of course, do not result in dopamine supersensitivity, because knockouts of some genes, such as those for adenosine A2A receptors, lead to dopamine subsensitivity [36,38]. In keeping with this reduction in dopamine sensitivity, the D2^{High} receptors are reduced by 75% in the striata of adenosine A2A knockout mice. Similarly, mice with knockouts of the metabotropic glutamate receptor-5 (mGluR5) are not supersensitive,
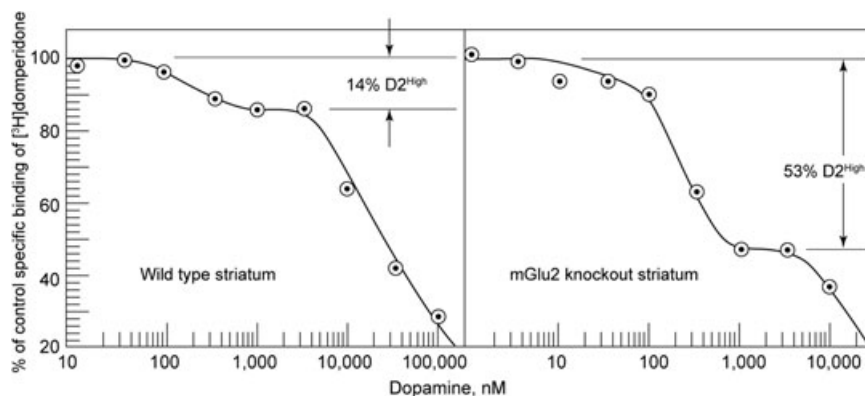
Figure 3 Left: Representative experiment on a control mouse striatal homogenate (average of duplicate measurements) showing competition between dopamine and [³H]domperidone for dopamine D2 receptors. The proportion of high-affinity D2 receptors, D2^{High}, was 14% in this tissue, with the average being $16.2 \pm 2.5\%$ in all the control tissues. The final concentration of [³H]domperidone was 2 nM. Nonspecific binding was defined by the presence of 1 μM S-sulpiride. Right: Representative experiment showing competition between dopamine and [³H]domperidone for dopamine D2 receptors in a striatal homogenate from an mGlu2 receptor knockout mouse. The proportion of high-affinity D2 receptors, D2^{High}, was 53% in this tissue. The final concentration of [³H]domperidone was 2 nM. (Reproduced from [39] with permission of Wiley-Liss, Inc.)

and the proportion of D2^{High} receptors does not increase (Figure 4).

Other Psychosis Models: Caesarian Birth with Anoxia, Psychostimulants, Social Isolation, Steroids

In another model for schizophrenia, adult rats that had been born by Caesarian section (with or without added anoxia) exhibit dopamine supersensitivity. Striata from these rats reveal a 2-fold to 5-fold elevation in the proportion of D2^{High} receptors (Figure 4), but no increase in the total population of D1 or D2 receptors [36,38].

Rats that have been sensitized by amphetamine, phencyclidine, quinpirole, or caffeine also become supersensitive to dopamine agonists. The striata from such supersensitive rats do not reveal any increase in dopamine D2 receptors, but show a 2-fold to 4-fold elevation in the proportion of D2^{High} receptors (Figure 4).

Social isolation is a risk factor for psychosis, and rats isolated from birth reveal dopamine supersensitivity and elevated D2^{High} receptors [40] (Figure 4).

Consistent with the hypothesis of D2^{High} being the convergent target for various psychoses is the fact that most psychoses respond to treatment with D2 antagonists. This includes phencyclidine psychosis [32,41]. The treatment of phencyclidine psychosis by haloperidol is significant, because haloperidol does not block NMDA receptors, indicating that the D2 target contributes to phencyclidine psychosis.

Measurement of D2^{High} Receptors in Humans

Because D2^{High} receptors are consistently elevated in the animal models of the various human psychoses, it appears reasonable to consider D2^{High} a common target for the convergence of the various psychosis pathways. Moreover, it is reasonable to hypothesize that risk factors or altered genes that lead to dopamine supersensitivity can also increase the risk for psychosis or schizophrenia.

The elevated D2^{High} receptors may be related to the clinical signs and symptoms of psychosis. Such a relation will need to be tested when the selective imaging of D2^{High} in patients becomes possible by radioactive D2^{High}-selective agonists [42].

Do D2^{High} States Exist *In Vivo*?

Despite data showing that high-affinity and low-affinity states of the D2 receptor can be detected in homogenized tissues, as well as the removal of D2^{High} by guanine nucleotides, the existence of D2^{High} states *in vivo* is less well established. For example, Sibley et al. [43] and Skinbjerg et al. [44] could not detect D2^{High} sites in intact cells, although such sites could be detected in intact cells by others [45]. In particular, Skinbjerg et al. [44] found that dopamine inhibited the binding of [³H]sulpiride at a single binding site in intact tissue culture cells, but only detected D2^{High} in homogenized cells with [³H]methylspiperone.
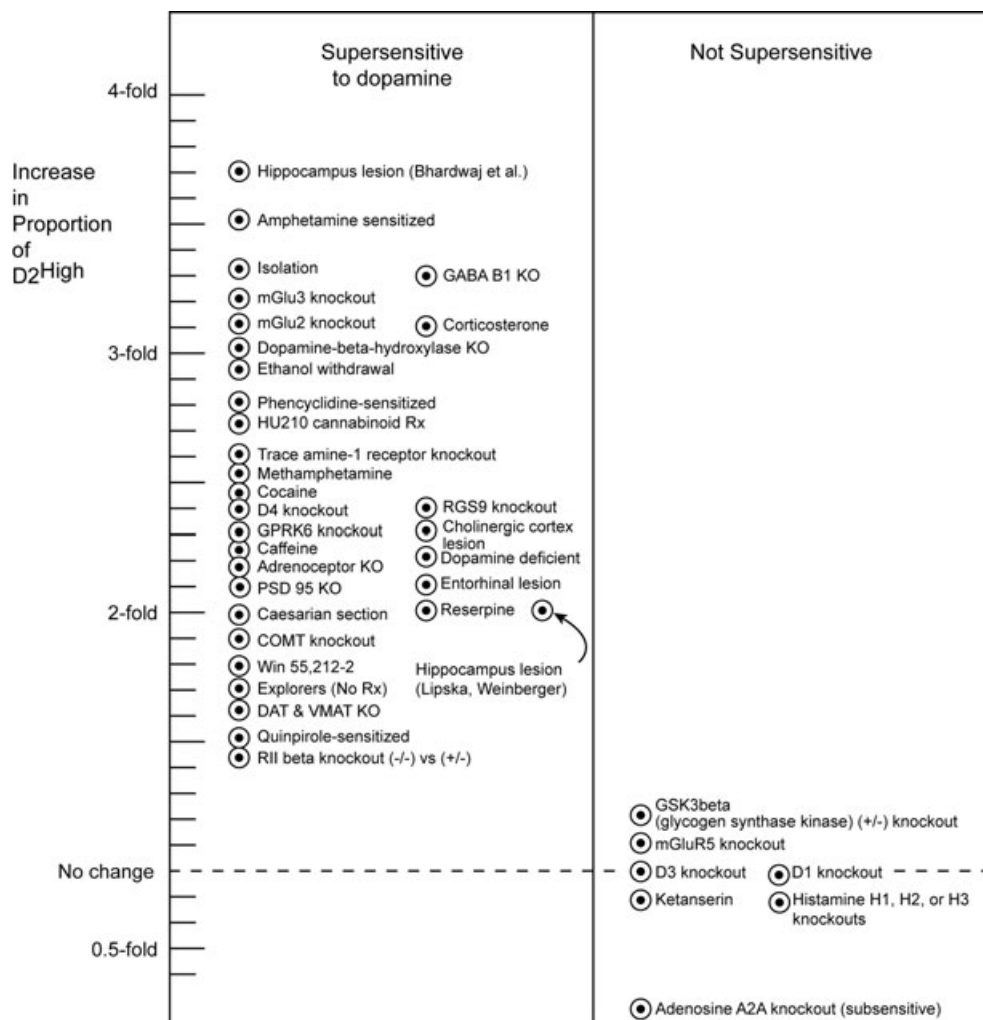
Figure 4 Animals that are supersensitive to dopamine-like drugs (e.g., apomorphine, cocaine, methylphenidate, amphetamine) reveal elevated proportions of dopamine D2 receptors that are in the high-affinity state for dopamine, D2^{High} (left). The data summarized here were obtained on striata from animals found to be dopamine supersensitive under the following conditions (listed from the upper left top down; unless otherwise specified, details are found in [36,38]: Mature rats with neonatal lesion of the hippocampus [46]. Rats sensitized by long-term treatment with amphetamine. Rats socially isolated after weaning [40]. Knockouts of GABA B1(–/–) receptors in mice (B. Bettler and P. Seeman, unpublished). Knockouts of metabotropic glutamate mGlu3 receptors in mice [39]. Knockouts of metabotropic glutamate mGlu2 receptors in mice [39]. Five days of 10 mg/kg corticosterone treatment to rats. Knockouts of the DBH (dopamine-beta hydroxylase) gene in mice. Long-term ethanol treatment of rats [47]. Rats sensitized to phencyclidine. Rats treated for 14 days with cannabinoid HU210 at 20 μg/kg (Moreno et al., 2005; F.J. Bermudez Silva, F. Rodriguez de Fonseca, J. Suarez, and P. Seeman, unpublished). Knockouts of trace amine-1 receptors in mice [48]. Rats sensitized by long-term treatment with methamphetamine [49]. Rats sensitized and addicted by long-term self-treatment with cocaine [50]. Knockouts of the RGS9–2 (regulator of G protein signaling-9) gene in mice. Knockouts of the dopamine D4 receptor gene in mice. Knockouts of the GPRK6 (G protein-coupled receptor kinase) gene in mice. Cholinergic le-

sion in the cerebral cortex of rats. Long-term high-dose treatment of rats with caffeine [51]. Mice made dopamine-deficient by tyrosine hydroxylase knockouts. Knockouts of alpha-1b-adrenoceptors in mice [52]. Rats with entorhinal lesions of the hippocampus [53]. Knockouts of PSD95 (postsynaptic density 95) gene in mice (M. Beaulieu, M. Caron, and P. Seeman, unpublished). Rats born by Caesarian section with anoxia. Rats treated with reserpine (5 mg/kg for 3 days; 2 days no drug). Mice with COMT (catechol-O-methyl transferase) gene knockouts. Rats with neonatal lesion of the hippocampus [54]. Rats treated with cannabinoid WIN 55,212–2 (4 mg/day for 14 days; F.J. Bermudez Silva, F. Rodriguez de Fonseca, J. Suarez, and P. Seeman, unpublished). Rats spontaneously active and explorative (no treatment)[55]. Mice with knockouts of dopamine transporter DAT or vesicle monoamine transporter-2 VMAT-2 [56]. Rats sensitized to quinpirole. Mice with knockouts of RIIbeta protein kinase A. The right side [36,38] shows either the lack of elevation, a minor elevation, or an actual fall in the proportion of D2^{High} receptors in mice with knockouts in the genes for glycogen synthase kinase (GSK3beta), metabotropic glutamate receptor mGluR5, dopamine D1 or D3 receptors, histamine H1, H2 or H3 receptors, and adenosine A2A receptors. Nine days of ketanserin treatment also had no effect on D2^{High} receptors. (Adapted and extended from [38]; with permission from Wiley & Sons, Inc.; Reproduced from [31] with permission from Walsh Medical Media LLC).

A search to detect D2[High] states *in vivo* by means of positron emission tomography is encountering inconsistencies. Intravenous amphetamine, for example, inhibited the binding of agonists (methoxy-NPA or radioactive (+)PHNO) more than it inhibited the binding of an antagonist (raclopride) [57,58].

However, Finnema et al. [59] found that apomorphine injected intravenously was about equally effective in displacing the agonist [11C]methoxy-NPA and the antagonist [11C]raclopride, data that suggest that there is no distinction between the binding of agonist and antagonist to D2 and that there may be no detectable D2[High] state *in vivo*. McCormick et al. [60,61] made a similar finding.

However, both Finnema et al. [59] and McCormick et al. [60,61] injected the cold agonist (apomorphine or NPA) intravenously 3 or 30 min before the intravenous injection of the radioligands. This procedure may result in an identical pattern of inhibition of the radioactive agonist and the radioactive antagonist.

In contrast, Ross and Jackson [62] simultaneously *co-injected* a dopamine agonist and the radioligand intravenously to measure D2 *in vivo*. By this method, Ross and Jackson successfully identified high-affinity and low-affinity D2 receptors occupied by the ligand but displaced by the agonist (such as pergolide), similarly to the pattern obtained *in vitro*.

Using the co-injection method, therefore, it was found that an intravenous injection of the agonist NPA inhibited the binding of [3H](+)PHNO more than the D2 antagonist [3H]raclopride [63], as measured *ex vivo*. This finding supports the idea that the sites for the binding of agonist and antagonist differ and that D2[High] sites exist *in vivo*. Overall, the greater inhibition (by 17%) of [3H](+)PHNO than [3H]raclopride by NPA suggests that the additional inhibition of 17% may reflect competition at D2[High] receptors [63]. This would agree with *in vitro* work that shows that between 10% and 20% of the D2 population exists in the D2[High] state [36,38]. In fact, the data of Finnema et al. [59] show that their lowest dose of 10 μg/kg apomorphine inhibited up to 15% more radioactive agonist than radioactive antagonist. This amount of 15% matches the proportion of D2 receptors that are normally in the D2[High] state.

The major biological difference between the pre-injection method [60,61] and the co-injection method [62,63] is that the co-injection method ensures that the arrival of the ligand and the NPA agonist occur at precisely the same moment. Considering that a receptor agonist can rapidly desensitize a receptor or internalize receptors within seconds or minutes [64,65], the arrival of NPA prior to that of [3H](+)PHNO would reduce the amount of D2[High] receptors available to [3H](+)PHNO.

Moreover, Seneca et al. [58] injected amphetamine 20 min before the radioligand; amphetamine-released dopamine may here contribute to desensitization or receptor internalization with consequent fewer D2[High] states. In addition, the protocol of Finnema et al. [59] may also lead to a rapid desensitization or receptor internalization by apomorphine with a reduction in D2[High] states in the 3 min before the agonist radioligand arrives.

Finally, the injection of a G-protein inhibitor directly into the striatum to block the G proteins that mediate dopamine-inhibited cyclase attenuates apomorphine-induced stereotyped behavior [66], demonstrating that the high-affinity state of D2 may be the functional state in the nervous system.

It is also possible to demonstrate directly that an agonist ligand such as [3H](+)PHNO actually binds to D2[High] sites *in vivo*. This is shown in Figure 6, where guanine nucleotide accelerated the *in vitro* release of [3H]PHNO that had been injected immediately before the demise of the animal, indicating that a significant amount of this ligand attached to D2[High] receptors *in vivo* moments before the striatum was removed. Note that the high-affinity state was reduced in matter of seconds by the nucleotide.

Multiple Pathways, Multiple Genes, Multiple Causes

If there are multiple neural pathways that mediate psychosis by converging onto a similar set of brain D2[High] targets, it suggests that there can be multiple causes and multiple genes associated with psychosis in general and schizophrenia in particular. It is even likely that different pedigrees have different sets of risk genes for schizophrenia.

Although the elevation of D2[High] receptors may be a necessary minimum for psychosis, it is not likely to be sufficient for full expression of psychotic features. For example, Hirvonen et al. [67] found D2 receptors elevated in healthy co-twins of schizophrenia individuals, suggesting that the elevation of D2 was necessary but not sufficient for psychosis to develop. The elevation of D2 is becoming recognized as a valuable biomarker for prognosis and outcome in first-episode psychosis [68].

While dopamine supersensitivity may be a basis for the positive signs and symptoms of psychosis, the biology underlying negative aspects of psychosis, such as cognition, is not known. Recent work, however, has found that overexpression of D2 in the striatum [69] leads to cognitive deficits in animals.
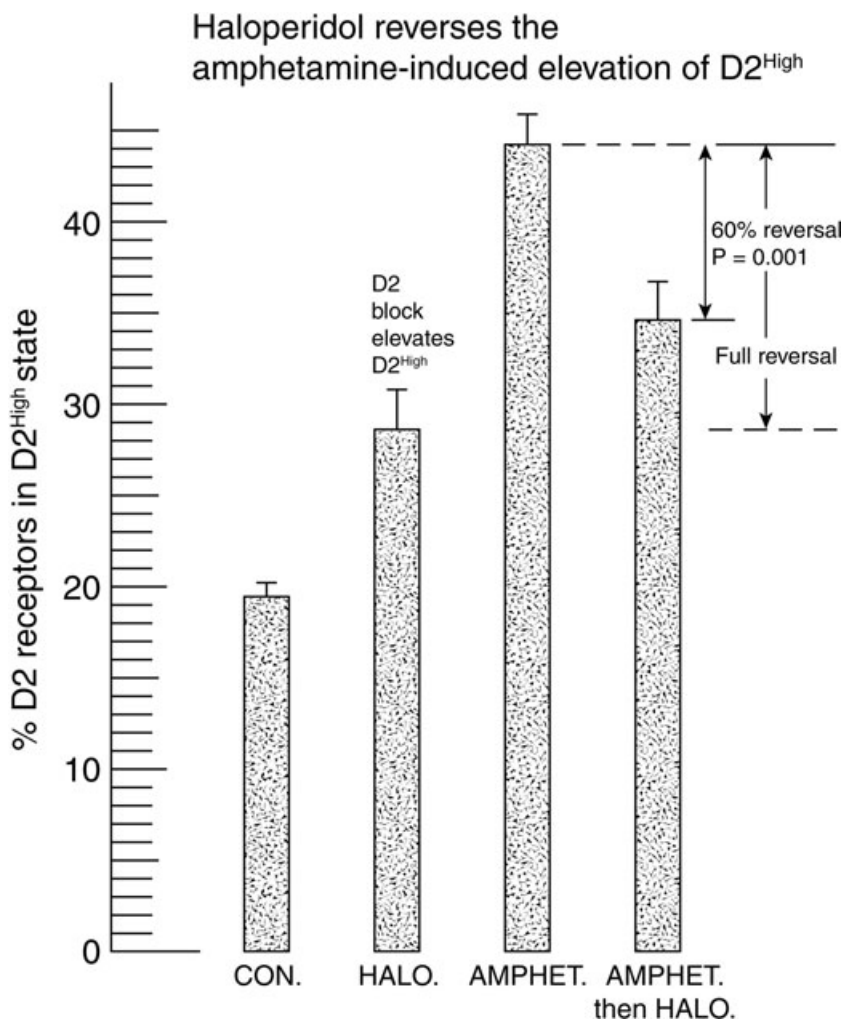
Haloperidol reverses the amphetamine-induced elevation of D2^{High}

Figure 5 Elevation of D2^{High} receptors by amphetamine, and reversal by haloperidol. The control level of D2^{High} receptors was $19.5 \pm 0.6\%$ (N = 20 rats). Haloperidol alone (0.25 mg/kg/day i.p. for 9 days) raised D2^{High} to $28.6 \pm 2\%$ (N = 6 rats), while amphetamine alone (1.6 mg/kg/day i.p. for 9 days followed by a drug holiday for 10 days) resulted in a D2^{High} level of $44.2 \pm 1.3\%$ (N = 6 rats). Haloperidol (0.25 mg/kg/day for 9 days), given after amphetamine, brought D2^{High} down to $34.8 \pm 1.6\%$, a reversal of 60%, where a full reversal would have corresponded to the level brought about by haloperidol alone. (Reproduced from [70] with permission of Elsevier Inc. and Copyright Clearance Center.)

Elevation of D2^{High} and the Dopamine Hypothesis of Schizophrenia

In summary, the overactivity of dopamine in schizophrenia [10,71] is supported by the following evidence, including the elevation of D2^{High} in animal models:

1. Antipsychotics, including partial agonist antipsychotics, reduce dopamine transmission by blocking D2 receptors in relation to their affinities for D2.
2. The reduction of dopamine transmission is relatively quick, a matter of a few days, and consistent with D2 blockade. Although patients with schizophrenia require 2 to 3 weeks before they are discharged from hospital, Delay et al. [72] observed that it only required about 3 days for chlorpromazine to attenuate the acute psychosis. Agid et al. [73,74] and Kapur et al. [75] report that antipsychotic clinical ef-

fects have their onset within the first 24 h of administration, and reach a peak of improvement within the first few days or first week, supporting earlier reports [76–82].
3. Although antipsychotics have different profiles of receptor blockade, they share a common action in blocking D2 receptors at the predicted concentrations in plasma water [22]. Moreover, antipsychotics such as remoxipride and amisulpiride are highly selective for D2 receptors, largely precluding the contribution of other receptors to the antipsychotic clinical action.
4. Antipsychotic occupancies of D2 are between 60% and 80% [83,84], with the exception of the aripiprazole which internalizes D2 receptors into the cytoplasm, and occupies D2 in excess of 90%. The antipsychotic occupancies of D3 and D4 receptors are highly variable, suggesting that these two receptors are not consistently targeted by antipsychotics [22].

Antipsychotics such as clozapine and quetiapine elicit negligible parkinsonism, even at relatively high doses. The blockade of serotonin-2 receptors or stimulation of serotonin-1 receptors may alleviate the parkinsonism of D2 blockade [85]. But when one calculates the ratio of the Ki values for D2 receptors divided by the Ki values for serotonin-2A or -1 receptors, there does not appear to be any obvious relation between parkinsonism and the ratio of the Ki values [31](ratios can be obtained from Table 1).

A factor may be the speed of dissociation of antipsychotics from D2. For example, all eight antipsychotics that result in low or negligible parkinsonism (remoxipride, clozapine, quetiapine, norclozapine, perlapine, S-(-)-amisulpiride, aripiprazole, and amoxapine) dissociate rapidly from the D2 receptor [22,86,87]. The attachment of clozapine, quetiapine, or amisulpiride to D2 is transient in the sense that these drugs quickly dissociate from D2 after 12 to 24 h. The patient's signs and symptoms, however, remain abated.

5. Although D2 receptors are elevated in postmortem brains of individuals who died with schizophrenia [20,35,88], findings with positron emission tomography in living patients did not find an elevation of D2 receptors [89,90]. These negative results do not invalidate the dopamine hypothesis of schizophrenia, because Hirvonen et al. [67] and Corripio et al. [68] show that D2 receptors are elevated in individuals with schizophrenia who have never been treated with antipsychotic drugs.

Although the elevation of D2^{High} in the animal models supports the dopamine hypothesis of schizophrenia, such D2^{High} receptors have yet to be detected in humans.

6. Of the various polymorphisms in D2, the variation at amino acid 311 in D2 was found associated with schizophrenia in a *meta*-analysis of 3,707 individuals [91,92]. Allen et al. [93] found the silent polymorphism at amino acid position Proline319Proline (nucleotide 957) in D2 to be associated with schizophrenia ($P < 0.00004$).

7. Selective overactivity of D2 in the striatum can also be the basis of cognitive difficulties [69].

8. As noted, further support for the dopamine hypothesis is that the majority of individuals with schizophrenia are supersensitive to the dopamine-like actions of amphetamine or methylphenidate [1] [see also supersensitivity psychosis in 94–100].

9. Antipsychotic clinical effects are clearly related to the occupancy of dopamine D2 receptors in the striatal region and not in the extra-striatal regions [101].

10. Although the binding of radioactive PHNO, a dopamine-like agonist, is normal in schizophrenia patients [102], this study only used a single brain scan and did not measure the "displaceable PHNO." The displaceable radio-PHNO can be determined using two brain scans per patient: the first brain scan is taken when injecting radio-PHNO alone, while the second brain scan entails co-injecting radio-PHNO and a low dose of apomorphine to block the attachment of radio-PHNO. The difference between the two scans would represent the binding of radio-PHNO to the D2^{High} receptors. This co-injection method has yet to be tried in control subjects and in individuals with schizophrenia.

11. It has been found that a glutamate agonist alleviates the signs and symptoms of schizophrenia [33], suggesting that there are nondopamine pathways to schizophrenia and to treatment [103]. However, these glutamate agonists, including LY404039, have a significant agonist affinity for D2^{High}, and would, therefore, be expected to have an aripiprazole-like
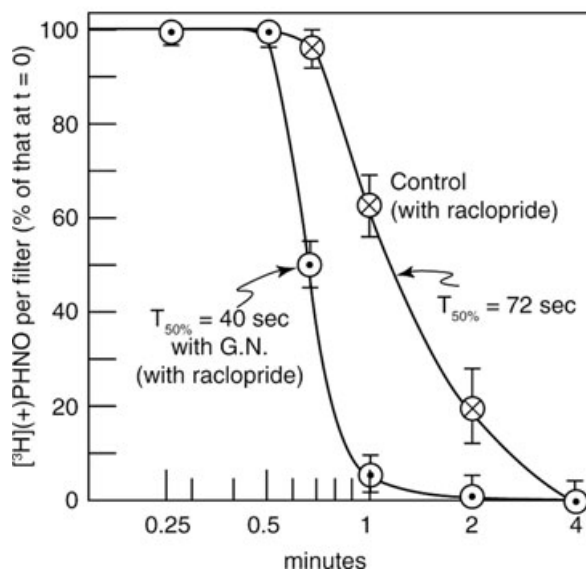

Figure 6 Showing that the agonist ligand, [3H](+)PHNO, binds to D2^{High} receptors *in vivo*. Ten μCi of [3H](+)PHNO was injected into the rat tail vein. Five minutes later, the brain striatum was removed, rapidly homogenized, and the [3H](+)PHNO allowed to dissociate (in the presence of 200 μM raclopride to prevent re-binding). The time for 50% dissociation of [3H](+)PHNO was 72 seconds. However, in the presence of 200 μM guanine nucleotide (GN) to convert the receptors into their D2Low state, the dissociation of [3H](+)PHNO was much more rapid with a 50% dissociation time of 40 seconds. The ability of GN to accelerate the release of [3H]PHNO indicated that a significant amount of this ligand had been attached to D2^{High} receptors *in vivo* moments before the striatum was removed [Seeman, unpublished].

Animal models of schizophrenia

Factors known to increase D2High by 200–400%

Mutations in genes for:

D4 receptor

GABA-B1 receptor

Trace amine receptor

Transporters

DBH, COMT, Nurr77

Postsynaptic density 95

RGS9-2, Gz

D2Low

D2High

Methamphetamine

Cannabis

Crack

Brain lesions

Birth injury

Social isolation

YOU'RE ROTTEN!

YOU'RE A PERVERT!

THEY'RE AFTER ME!

WITHDRAWS FROM:
STUDIES
FRIENDS
SPORTS

FLOODED WITH THOUGHTS
CAN'T CONCENTRATE
FEELS MYSTICAL POWERS
FEARS FOOD POISONING
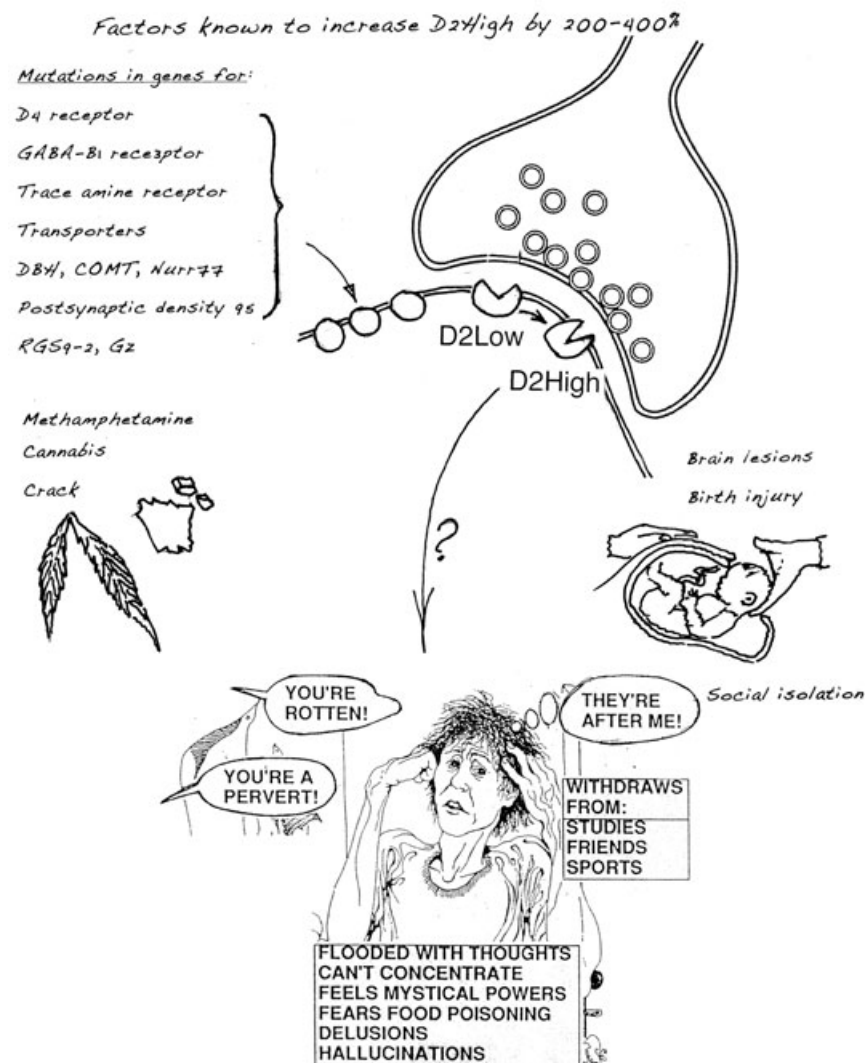DELUSIONS
HALLUCINATIONS

Figure 7 Summary of biological roads to schizophrenia [104]. Many risk factors lead to elevated D2^{High} receptors and dopamine supersensitivity that underly signs and symptoms of schizophrenia (from [104], reproduced with permission from SZ Publications).

agonist action [32]. Although Fell et al. [103] did not find a dopamine *in vitro* component of action for mGluR2 agonists, as compared to other studies [32,34], the tissue membranes used by Fell et al. were extensively washed, a procedure known to cause a major loss of dopamine receptors [Refs. in 32 and 34].

12. Although it is known that long-term use of antipsychotics can lead to tardive dyskinesia, dopamine supersensitivity, and antipsychotic-induced supersensitivity psychosis [94–100], antipsychotics can reverse the elevation of D2^{High}, as shown in Figure 5. The development of tardive dyskinesia depends on the long-term accumulation of the antipsychotic in the neuromelanin of the substantia nigra, with

secondary injury to the nigral cells and axon terminal sprouting [105].

Finally, a summary of the factors elevating D2^{High} and possibly leading to schizophrenia is shown in Figure 7.

Conflict of Interest

The author is also affiliated with Clera, Inc., a pharmaceutical company, but has no other competing interests.

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