

Dopamine supersensitivity correlates with D2^{High} states, implying many paths to psychosis

Philip Seeman^{a,b}, David Weinshenker^c, Remi Quirion^d, Lalit K. Srivastava^d, Sanjeev K. Bhardwaj^d, David K. Grandy^e, Richard T. Premont^f, Tatyana D. Sotnikova^g, Patricia Boksa^d, Mufida El-Ghundi^a, Brian F. O'Dowd^a, Susan R. George^a, Melissa L. Perreault^h, Pekka T. Männistöⁱ, Siobhan Robinson^j, Richard D. Palmiter^{i,k}, and Teresa Tallerico^l

^aPharmacology Department, Medical Science Building, Room 4344, and ^bPsychiatry Department, Medical Science Building, Room 4343, University of Toronto, 1 King's College Circle, Toronto, ON, Canada M5S 1A8; ^cDepartment of Human Genetics, Emory University School of Medicine, Whitehead Building, Suite 301, 615 Michael Street, Atlanta, GA 30322; ^dDepartment of Psychiatry, McGill University, Douglas Hospital Research Centre, 6875 LaSalle Boulevard, Verdun, Montreal, QC, Canada H4H 1R3; ^eDepartment of Physiology and Pharmacology, Oregon Health and Science University, Portland, OR 97239; ^fDepartment of Medicine (Gastroenterology), Liver Center, 329 Sands Building, Campus Box 3083, and ^gDepartment of Cell Biology, 487 Carl Building, Box 3287, Duke University Medical Center, Durham, NC 27710; ^hDepartment of Psychiatry and Behavioral Neurosciences, McMaster University, 1200 Main Street West, Health Science Centre, Room 4N82, Hamilton, ON, Canada L8N 3Z5; ⁱPharmacology and Toxicology Department, University of Kuopio, P.O. Box 1627, Kuopio 70211, Finland; ^jNeurobiology and Behavior Program, Department of Psychology, University of Washington, Seattle, WA 98195; and ^kHoward Hughes Medical Institute, Box 357370, University of Washington, Seattle, WA 98195

Contributed by Richard D. Palmiter, January 10, 2005

Dopamine supersensitivity occurs in schizophrenia and other psychoses, and after hippocampal lesions, antipsychotics, ethanol, amphetamine, phencyclidine, gene knockouts of *Dbh* (dopamine β -hydroxylase), *Drd4* receptors, *Gprk6* (G protein-coupled receptor kinase 6), *Comt* (catechol-O-methyltransferase), or *Th*^{-/-}, *Dbh*^{Th/+} (tyrosine hydroxylase), and in rats born by Cesarean-section. The functional state of D2, or the high-affinity state for dopamine (D2^{High}), was measured in these supersensitive animal brain striata. Increased levels and higher proportions (40–900%) for D2^{High} were found in all these tissues. If many types of brain impairment cause dopamine behavioral supersensitivity and a common increase in D2^{High} states, it suggests that there are many pathways to psychosis, any one of which can be disrupted.

addiction | dopamine receptors | gene knockouts | schizophrenia

Psychotic symptoms occur in many diseases, including schizophrenia and prolonged drug abuse. Although many chromosome regions and genes have been found associated with schizophrenia (1, 2), no single gene of major effect has yet been identified. Nevertheless, regardless of the causes of psychosis, antipsychotic drugs are mostly effective in alleviating the symptoms. The clinical antipsychotic potencies of these drugs are directly related to their affinities for the dopamine D2 receptor (3, 4), suggesting that the properties of this receptor are disturbed in psychosis. It is uncertain whether the total density of D2 receptors in schizophrenia is elevated (5, 6). The more relevant question, however, is whether the functional state of D2, or the state of high-affinity for dopamine, D2^{High} (7), is elevated, and this has not been investigated in schizophrenia or in any of the psychoses. An elevated density of D2^{High} would explain why up to 70% of individuals with schizophrenia are supersensitive to dopamine (8), but supersensitivity may have other bases. Therefore, it is important to determine the causes of dopamine supersensitivity (i.e., behavioral supersensitivity to dopamine-mimetics). Experimentally, dopamine supersensitivity occurs after a neonatal hippocampal lesion (9), long-term antipsychotics (10), ethanol or amphetamine (11), in gene knockouts of *Dbh* (dopamine β -hydroxylase) (12), *Drd4* dopamine receptors (13), *Gprk6* (G protein-coupled receptor kinase 6) (14), *Comt* (catechol-O-methyltransferase) (15, 16), or *Th*^{-/-}, *Dbh*^{Th/+} (tyrosine hydroxylase, dopamine-deficient) (17–19), and in rats born by Cesarean section (20). Although antipsychotics are known to elevate the density of dopamine D2 receptors by $\approx 25\%$ above control levels, no such elevations occur in ethanol withdrawal (21), amphetamine-sensitized animals (22), *Gprk6* or *Comt* knockouts (14, 15), dopamine deficient mice (18), or rats born by Cesarean section (20).

The basis of supersensitivity to amphetamine or dopamine agonists thus remains puzzling. However, it has recently been found

that, despite the absence of any elevation in total dopamine D2 receptors in the striata of amphetamine-sensitized animals, there is a dramatic 360% increase (22) in the density of D2^{High} states (23). Therefore, we thought it important to examine whether D2^{High} would also be invariably elevated in other conditions showing dopamine supersensitivity. We found this to be the case in studying the striata from many types of animals that are known to be dopamine supersensitive after treatment with either antipsychotics, quinpirole, ethanol, or amphetamine, after a hippocampal lesion, or after five types of gene knockouts.

Materials and Methods

Antipsychotic Treatment. Adult male Sprague–Dawley rats, weighing 200–225 g at the start of the experiment, were used. For 9 days, the animals received daily i.p. injections (0.5 ml) of saline (0.9%), haloperidol (0.045 mg/kg), risperidone (0.75 mg/kg), olanzapine (0.75 mg/kg), clozapine (35 mg/kg), or quetiapine (25 mg/kg). These doses occupy 70% of brain dopamine D2 receptors in rats, a level of occupancy associated with human clinical response to antipsychotics in schizophrenia (24). The nonantipsychotic ketanserin was used as a comparison drug and given i.p. at 15 mg/kg for 9 days.

Amphetamine Sensitization. The procedure for sensitizing rats to amphetamine has been published (22).

Ethanol Treatment and Withdrawal. Ethanol was given as follows: 2 g/kg i.p. twice daily; i.e., 1.4 ml of 18% ethanol in 0.9% NaCl per 100 g at 9 a.m. and again at 3 p.m. daily to rats for 10 days.

Hippocampal Lesion. The procedure for lesioning the rat hippocampus has been described (9).

Gene Knockouts (Homozygous). Gene knockouts for the *Dbh* gene (12) were developed in C57BL/6J $\times$ 129/SvEv mice. Knockouts for the *Drd4* receptor gene (13) and the *Gprk6* gene were developed in the C57BL/6J $\times$ 129/SvJ strain of mice. *Comt*-deficient male C57BL/6J mice and dopamine-deficient mice were generated and genotyped as described (15–19). The dopamine-deficient mice required daily L-DOPA (50 mg/kg i.p.) for survival (yet maintaining dopamine supersensitivity), and the animals were killed 24 h after the last dose of L-DOPA. Congenic mice lacking *Drd1a* (backcrossed to C57BL for 11 generations) were prepared (25).

Freely available online through the PNAS open access option.

Abbreviation: GN, guanylimidodiphosphate.

^bTo whom correspondence should be addressed. E-mail: philip.seeman@utoronto.ca.

© 2005 by The National Academy of Sciences of the USA

Rats Born by Cesarean Section. The procedure for obtaining rats by cesarean section with or without anoxia are given elsewhere (20).

Quinpirole or Phencyclidine Sensitization. Adult rats received 13 doses of quinpirole (0.05 mg/kg), followed by six doses (0.5 mg/kg) s.c. twice weekly. After the series of injections, the rats had an enhanced locomotor response to quinpirole (up to 1 mg/kg). Rats were sensitized to phencyclidine (Sigma; 2.5 mg/kg/day i.p. for 4 days, followed by 7 days without drug).

[³H]Ligands. [³H]Raclopride (60–80 Ci/mmol) was from PerkinElmer Life Sciences (Boston). [³H]Domperidone was custom synthesized as [phenyl-³H(N)]domperidone (42 Ci/mmol) by PerkinElmer Life Sciences, and used at a final concentration of 1.2–3 nM for competition with dopamine.

Saturation of Dopamine D2 Receptors by [³H]Raclopride (Scatchard Analysis). The frozen striata were blotted and weighed frozen. Buffer was added (50 mM Tris-HCl, pH 7.4/1 mM EDTA/5 mM KCl/1.5 mM CaCl₂/4 mM MgCl₂/120 mM NaCl) to yield 4 mg of tissue per ml. The method for determining the density of D2 receptors has been reported (21–23). Nonspecific binding for

dopamine D2 receptors was defined as that in the presence of 10 μ M S-sulpiride. The density of [³H]raclopride binding sites and the dissociation constant (K_d) were obtained by Scatchard analysis.

Competition Between Dopamine and [³H]Raclopride or [³H]Domperidone. The competition between dopamine and [³H]raclopride or [³H]domperidone for binding at the dopamine D2 receptors was done as reported (23).

Statistics. The competition data were analyzed by using a program that provided two statistical criteria to judge whether a two-site fit was better than a one-site fit, or whether a three-site fit was better than a two-site fit (ref. 21 and references therein).

Results

Long-Term Antipsychotic Treatment. Three methods were used to detect the D2^{High} states *in vitro*. The first method was a dopamine/[³H]raclopride competition experiment in the presence of low NaCl (10 mM) (21, 22). The low NaCl was used because high-affinity states were not detected by dopamine/[³H]raclopride competition in 120 mM NaCl, as shown in Fig. 1A. A second method was to use dopamine/[³H]domperidone compe-

Table 1. Dopamine supersensitivity in striatum: Increased D2^{High} receptors

	n*	D2 density, pmol/g (nM)	Total D2 density, pmol/g (nM) [†]	D2 ^{High} , pmol/g [†]	Fold differential [‡]	Dopamine/ [³ H]raclopride		Dopamine/ [³ H]domperidone	
						% D2 ^{High} (n = 2),* %	Increase in D2 ^{High} proportion	% D2 ^{High} (n = 2),* %	Increase in D2 ^{High} proportion
Rat control striata	34	19.8 $\pm$ 0.7 (1.4 $\pm$ 0.1)	21.7 $\pm$ 0.7 (1.46 $\pm$ 0.1)	1.94 $\pm$ 0.2	Control	10–28	Control	10–20	Control
Ketanserin, 9 days	2	13.4 $\pm$ 0.5 (1.8)	15 $\pm$ 0.6 (1.8)	1.6 $\pm$ 0.3	0.83-fold	26	Same as control	ND	ND
Haloperidol, 9 days	2	15.8 $\pm$ 1.8 (1.1)	20.5 $\pm$ 1.6 (1.3)	4.7 $\pm$ 0.35	2.4-fold	43 $\pm$ 5	1.9-fold	44	2.3-fold
Clozapine, 9 days	2	13.9 $\pm$ 0.5 (1.3)	18.9 $\pm$ 0.7 (1.4)	5 $\pm$ 0.6	2.6-fold	39 $\pm$ 10	1.7-fold	36	1.9-fold
Olanzapine, 9 days	2	11.9 $\pm$ 0.6 (1.86)	16 $\pm$ 0.5 (1.8)	4.1 $\pm$ 0.6	2.1-fold	55 $\pm$ 12	2.4-fold	40	2.1-fold
Risperidone, 9 days	2	13.3 $\pm$ 0.5 (1.9)	18.3 $\pm$ 0.6 (2.1)	5 $\pm$ 0.6	2.6-fold	33 $\pm$ 8	1.6-fold	60	3.2-fold
Quetiapine, 9 days	2	12.8 $\pm$ 0.4 (1.4)	16.8 $\pm$ 0.7 (1.4)	4 $\pm$ 0.7	2.1-fold	49 $\pm$ 10	2.1-fold	26	1.4-fold
Ethanol withdrawal	8	19 $\pm$ 0.8 (1.5)	26.1 $\pm$ 0.8 (2)	7.2 $\pm$ 0.6	3.7-fold	33 (n = 1)	3-fold	ND	ND
Hippocampus lesion	3	12.1 $\pm$ 4 (1.5)	20 $\pm$ 5 (1.8)	7.9 $\pm$ 0.9	4.1-fold	17 (n = 3)	1.7-fold	37 (n = 3)	3.7-fold
Amphetamine sensitized	2	19.3 $\pm$ 0.7 (2.3)	25.3 $\pm$ 0.6 (2.7)	6 $\pm$ 0.7	3.1-fold	38 (n = 1)	3.5-fold	ND	ND
Vaginal birth (control)	3	12.6 $\pm$ 0.4 (2.3 $\pm$ 0.3)	13.7 $\pm$ 0.2 (1.5 $\pm$ 0.2)	1.1 $\pm$ 0.3	Control	10 $\pm$ 2	Control	16 $\pm$ 2	Control
Cesarean section	3	10.3 $\pm$ 0.6 (1.3 $\pm$ 0.1)	16.3 $\pm$ 0.9 (1.1 $\pm$ 0.1)	6.1 $\pm$ 0.3	5.6-fold	27 $\pm$ 3	2.7-fold	32 $\pm$ 3	2-fold
Cesarean section + anoxia	3	12.9 $\pm$ 0.7 (1.5 $\pm$ 0.2)	18.4 $\pm$ 1.2 (1.5 $\pm$ 0.1)	5.5 $\pm$ 0.7	5-fold	27 $\pm$ 3	2.7-fold	36 $\pm$ 3	2.3-fold
Control striata	2	17.3 $\pm$ 3.2 (2)	18.5 $\pm$ 3 (1.9)	1.15 $\pm$ 0.25	Control	28 $\pm$ 4	Control	14 $\pm$ 4	Control
Gprk6 knockout	2	15.2 $\pm$ 3.2 (0.8)	20.3 $\pm$ 3.4 (1.1)	5.1 $\pm$ 0.2	4.4-fold	46 $\pm$ 5	1.6-fold	32 $\pm$ 6	2.3-fold
Control striata	2	16.2 $\pm$ 0.2 (1.98)	16.8 $\pm$ 0.3 (1.8)	0.6 $\pm$ 0.1	Control	25 $\pm$ 5	Control	18 $\pm$ 4	Control
Drd4 knockout	2	14.8 $\pm$ 0.9 (1.6)	20.7 $\pm$ 0.9 (2)	5.95 $\pm$ 0.1	9.9-fold	48 $\pm$ 5	1.9-fold	42 $\pm$ 4	2.3-fold
Control striata	2	14.9 $\pm$ 0.9	17.5 $\pm$ 0.4	2.6 $\pm$ 0.5	Control	16 $\pm$ 5	Control	10 $\pm$ 4	Control
Dbh knockout	3	15.3 $\pm$ 1.3	23.5 $\pm$ 1.7	8.3 $\pm$ 3	3.2-fold	30 $\pm$ 5	1.9-fold	30 $\pm$ 5	3-fold
Control striata	4	ND	ND	ND	ND	ND	ND	22 $\pm$ 3	Control
Comt knockout	4	ND	ND	ND	ND	ND	ND	42 $\pm$ 3	1.9-fold
Control striata	2	ND	ND	ND	ND	ND	ND	14 $\pm$ 2	Control
Drd1a knockout	2	ND	ND	ND	ND	ND	ND	14 $\pm$ 2	Same as control
Control striata	5	ND	ND	ND	ND	ND	ND	13.6 $\pm$ 3.6	Control
Quinpirole-sensitized	9	ND	ND	ND	ND	ND	ND	21.8 $\pm$ 3.2	1.6-fold
Control nucleus accumbens	5	ND	ND	ND	ND	ND	ND	19 $\pm$ 3	Control
Quinpirole-sensitized	9	ND	ND	ND	ND	ND	ND	28 $\pm$ 3	1.5-fold
Control striata	8	ND	ND	ND	ND	ND	ND	12.5 $\pm$ 8.5	Control
Phencyclidine-sensitized	8	ND	ND	ND	ND	ND	ND	34.5 $\pm$ 4	2.8-fold
Control striata	6	ND	ND	ND	ND	ND	ND	13.7 $\pm$ 1.1	Control
Dopamine-deficient	6	ND	ND	ND	ND	ND	ND	30 $\pm$ 3.1	2.2-fold

Scatchard experiments (first two columns) contained 120 mM NaCl. Competition experiments for dopamine/[³H]raclopride contained 10 mM NaCl, and those for dopamine/[³H]domperidone contained 120 mM NaCl. ND = not done; $\pm$ indicates SE. Numbers in parentheses in first two columns are [³H]raclopride K_d values.

*n = 2 independent experiments unless stated otherwise.

[†]D2^{High} calculated as Total D2 density – D2 density.

[‡]Fold differential calculated as D2^{High}/control D2^{High}.

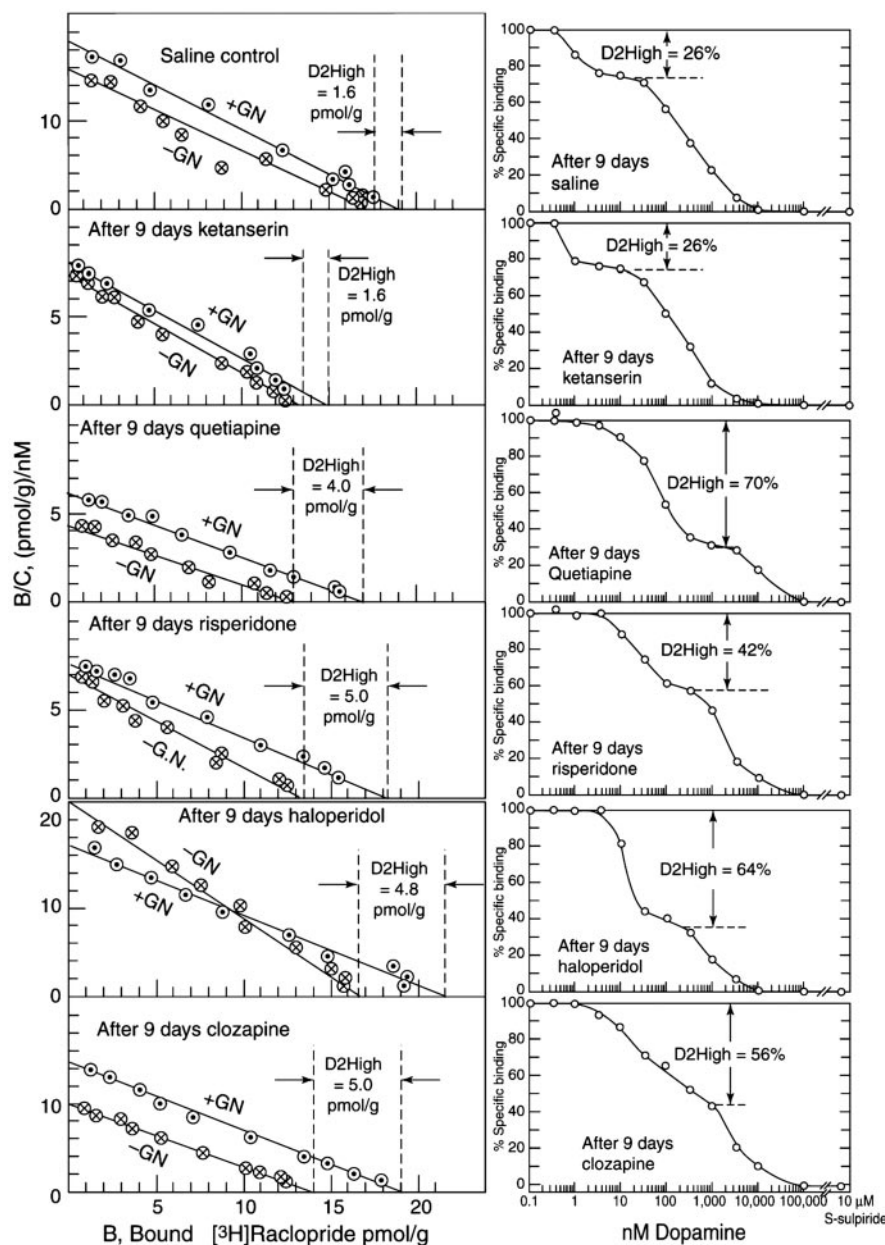


Fig. 2. Increase in $D2^{High}$ states after 9 days of antipsychotic treatment. (Left) $[^3H]$ Raclopride saturation method (additional details in Fig. 1). (Right) Dopamine/ $[^3H]$ raclopride competition method. Representative experiments are shown (Table 1).

shown elsewhere (23), dopamine (with its K_d of 1.75 nM) is not effective in competing versus the much more tightly bound $[^3H]$ spiperone (with its K_d of 60 pM), especially in 120 mM NaCl.

It is not known whether the saturation method and the two competition methods reveal the same population of $D2^{High}$ states. It is likely that the saturation method reveals $D2^{High}$ states that are normally occluded or occupied by endogenous dopamine. However, the competition method may reveal $D2^{High}$ states that are either occupied or not occupied by dopamine. Further work will be needed to examine this.

It is surprising that these diverse impairments of the brain (drugs, lesions, gene knockouts, cesarean sections) all resulted in a common $D2^{High}$ basis for dopamine supersensitivity, especially surprising in cases where no direct interference with dopamine transmission was made. It is possible that this shift to more $D2^{High}$ states is a nonspecific reaction to brain impairment, making the animal

more responsive to a change in its environment. However, the *Drd1a* knockout data (with reduced sensitivity to amphetamine and normal proportions of $D2^{High}$ states) indicate that the knockout process does not elicit a nonspecific increase in sensitivity to amphetamine or in $D2^{High}$ states.

Although the sensitization procedures were also associated with small increases in the total population of D2 receptors, these increases were especially small compared to the elevations found in the $D2^{High}$ states. For example, compared to controls (in the presence of guanylimidodiphosphate), the total D2 density went up by 10% in *Gprk6* knockouts, 23% in *Drd4* knockouts, and 34% in *Dbh* knockouts, in contrast to the elevations of 3.2- to 9.9-fold in the $D2^{High}$ component. In the case of the cesarean section rats, previous work (20) did not reveal a significant rise in the total D2 population, a situation similar to that found in ethanol withdrawal or after amphetamine sensitization (21, 22).

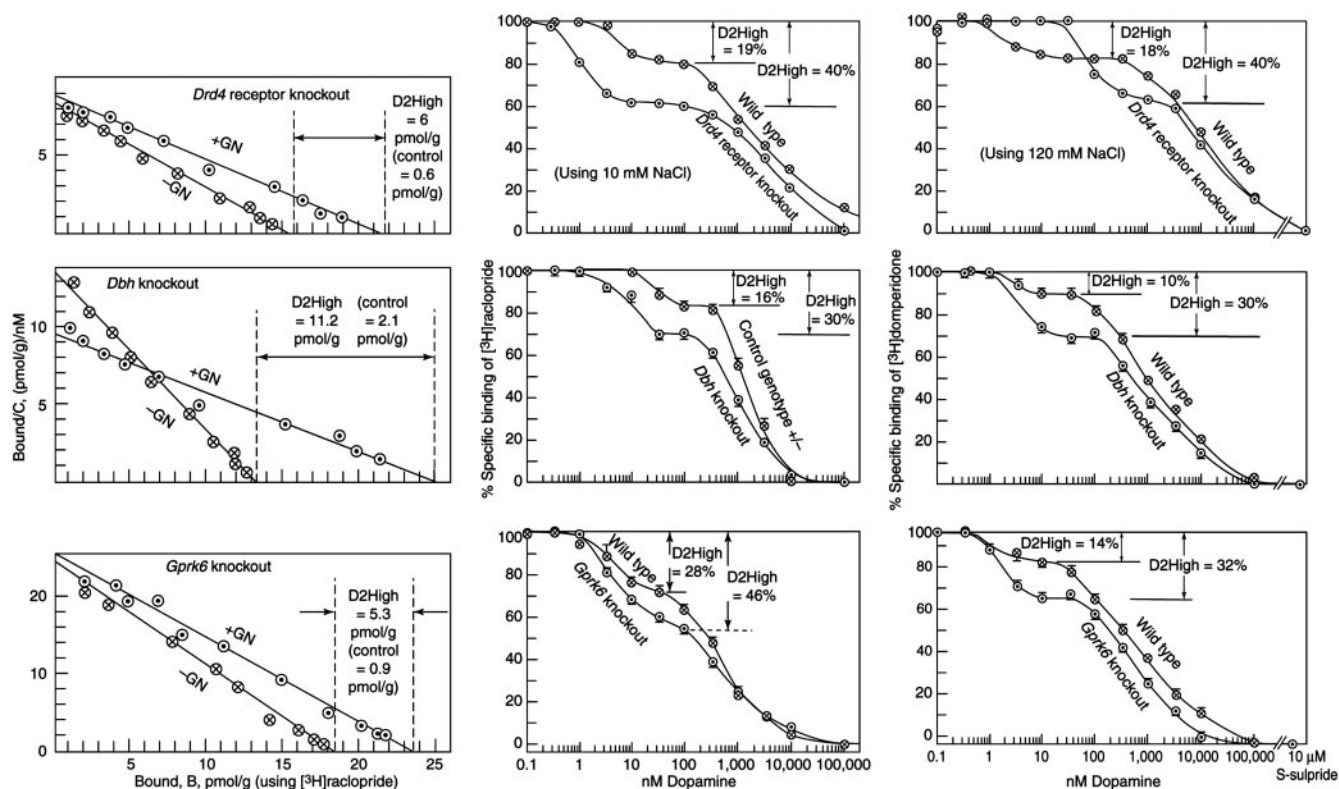


Fig. 3. Increase in $D2^{High}$ states in gene knockouts for the *Drd4* dopamine receptor, *Dbh*, and *Gprk6*. (Left) $[^3H]$ Raclopride saturation method (see Fig. 1). (Center) Dopamine/ $[^3H]$ raclopride competition method. (Right) Dopamine/ $[^3H]$ domperidone competition method. Representative experiments are shown, bars indicate SE (Table 1).

All of the animals in this study are known to be supersensitive to amphetamine or dopamine agonists with the exception of the *Drd1a* knockout mice (26), which revealed a normal proportion of $D2^{High}$

states. The present data suggest that the sensitivity to amphetamine may be related to the magnitude of the $D2^{High}$ states. However, the converse may not hold. That is, although the present data support

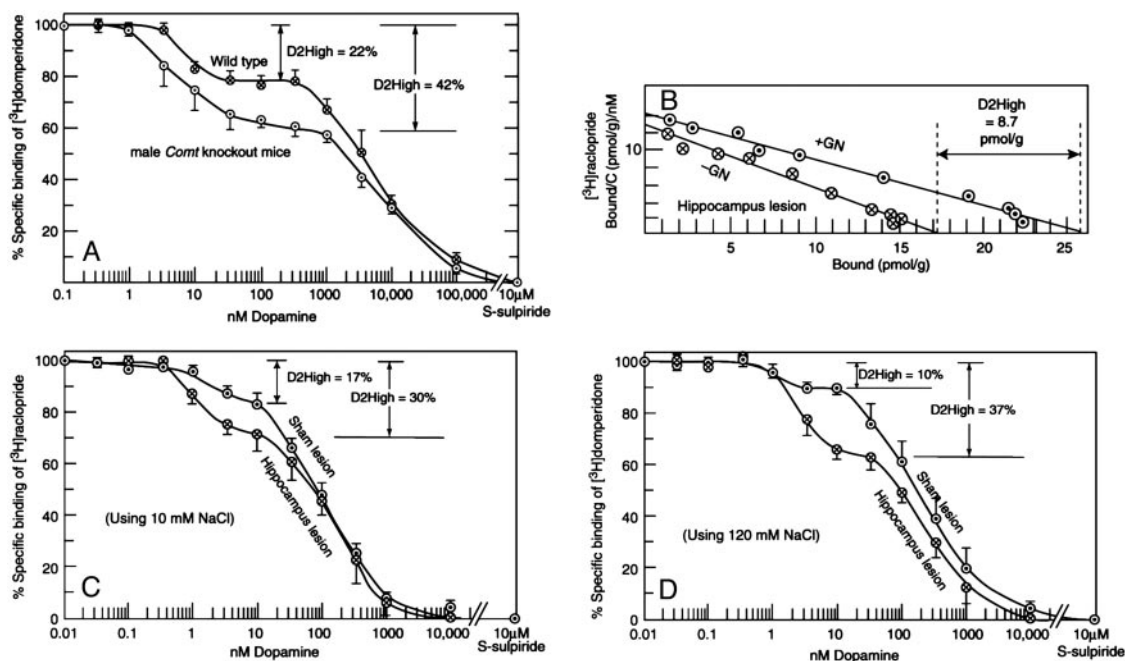


Fig. 4. Increased $D2^{High}$ states in knockouts and lesions. (A) Increase in $D2^{High}$ states in gene knockouts for *Comt*, using the dopamine/ $[^3H]$ domperidone competition method ($n = 4 \pm SE$; Table 1). (B) Increase in $D2^{High}$ states in striata from adult animals that had been lesioned in the hippocampus at an early age. $[^3H]$ Raclopride saturation method; control value was 2 pmol/g (see Table 1). (C) Dopamine/ $[^3H]$ raclopride competition method. (D) Dopamine/ $[^3H]$ domperidone competition method. Bars indicate SE ($n = 3$; Table 1).

