# Positron Emission Tomography Reveals Elevated $D_2$ Dopamine Receptors in Drug-Naive Schizophrenics

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**In postmortem studies of patients with schizophrenia, $D_2$ dopamine receptors in the basal ganglia have been observed to be more numerous than in patients with no history of neurological or psychiatric disease. Because most patients with schizophrenia are treated with neuroleptic drugs that block $D_2$ dopamine receptors in the caudate nucleus, it has been suggested that this increase in the number of receptors is a result of adaptation to these drugs rather than a biochemical abnormality intrinsic to schizophrenia. With positron emission tomography (PET), the $D_2$ dopamine receptor density in the caudate nucleus of living human beings was measured in normal volunteers and in two groups of patients with schizophrenia—one group that had never been treated with neuroleptics and another group that had been treated with these drugs. $D_2$ dopamine receptor densities in the caudate nucleus were higher in both groups of patients than in the normal volunteers. Thus, schizophrenia itself is associated with an increase in brain $D_2$ dopamine receptor density.**

SEVERAL LINES OF EVIDENCE LINK the dopaminergic neurotransmitter system to schizophrenia. The antipsychotic action of neuroleptic drugs (*1*) is correlated with the blockade of $D_2$ dopamine receptors (*2*). Amphetamines, which elevate synaptic dopamine levels, can induce psychotic states resembling schizophrenia and exacerbate symptoms of schizophrenic patients (*3*). Increased numbers of $D_2$ dopamine receptors have been detected in postmortem studies of the brains of schizophrenic patients, while the numbers of $D_1$ dopamine receptors were unchanged (*4*) (*5*). In some studies, these increases were attributed to prior neuroleptic treatment of the patients (*4*), while in other studies increases were found in drug-free schizophrenic patients (*5*). However, chronic neuroleptic treatment of animals can elevate $D_2$ dopamine receptor density even after neuroleptic withdrawal (*6*). Hence, the interpretation of elevation in dopamine receptors in postmortem tissue has remained controversial. We therefore studied $D_2$ dopamine receptors in vivo with positron emission tomography (PET) in two groups of schizophrenic patients—one previously treated and another never treated with neuroleptics. With PET scanning, we could quantitate neurotransmitter receptor density and affinity in the brains of living human subjects. We used (3-*N*-[$^{11}$C]methyl)spiperone ([$^{11}$C]NMSP) as the radioligand; others have used [$^{11}$C]chlorpromazine, [$^{11}$C]raclopride, [$^{76}$Br]spiperone, 3-*N*-methyl-[$^{18}$F]-spiperone, [$^{18}$F]spiperone, 3-(2′-[$^{18}$F]fluoroethyl)spiperone, and *N*-(3-[$^{18}$F]fluoropropyl)spiperone (*7*).

We used two PET scans (*8*) to estimate caudate $D_2$ dopamine receptor densities. The first scan was taken when the receptors were not blocked. The second scan, which was preceded by the administration of the unlabeled $D_2$ dopamine receptor blocking drug haloperidol, revealed binding in the blocked state (Fig. 1) (*9*). Eleven normal volunteers (nine males and two females), ten drug-naive (eight males and two females), and five previously treated (all males) schizophrenic patients were studied (Table 1). All subjects gave informed consent in compliance with the Johns Hopkins Human Investigation Committee. None of the ten drug-naive schizophrenics received any neuroleptic therapy prior to the first PET scan. Eight of the ten received a single dose of 7.5 mg of haloperidol before the second PET scan, as did the normal volunteers. Two of the ten received intermittent doses of haloperidol for 1 month before the second PET scan. The average duration of illness in the ten drug-naive patients (± SD) was 5 ± 3 years. The previously treated schizophrenics were neuroleptic-free for a minimum of half a month (average 2.6 ± 2.5 months) before the PET studies. Their average duration of illness was 7 ± 2 years. All patients were diagnosed by a research psychiatrist on the basis of at least 2 hours of interviewing. They have been followed subsequently; in no instance has the diagnosis been changed. All of the patients met the Diagnostic and Statistical Manual (third edition) (DSM III) criteria for the diagnosis of chronic schizo-

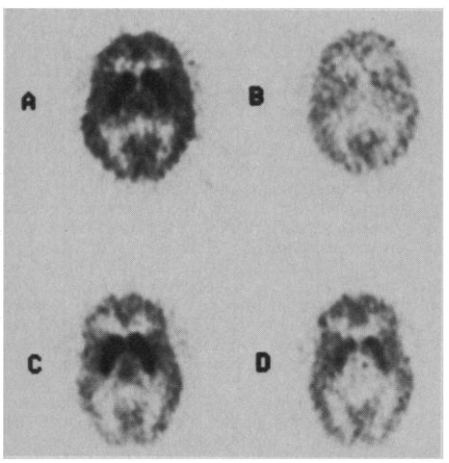


Fig. 1. PET scan images of the radioactivity distribution obtained at the level of the caudate nucleus and putamen 65 to 95 minutes after injection of [$^{11}$C]NMSP in a normal subject (A and B) and in a schizophrenic patient (C and D) in the unblocked (A and C) and the blocked state (B) and (D). Both subjects were males, 24 years of age and received a single dose of 7.5 mg of haloperidol before the second PET scan. This illustrates the more pronounced blockade of [$^{11}$C]NMSP uptake in the caudate and putamen in the normal subject as compared to the patient, despite the fact that serum haloperidol was lower in the normal (2.5 ng/ml) than in the patient (4 ng/ml). $B_{max}$ values in the normal and the schizophrenic subjects were 11.5 and 36.2 pmol/g, respectively.

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phrenia (*10*). A careful history of previous drug exposure was obtained for each patient. A patient was considered drug-naive if the patient's history (obtained from each patient and one relative who resided with the patient), both at the time of PET scan and within 2 to 4 weeks following PET scan, indicated no treatment (*11*). Psychiatric ratings used to characterize psychopathology included the Brief Psychiatric Rating Scale (BPRS), a modified version of the Present State Examination (Mini PSE), the Schedule for the Assessment of Negative Symptoms (SANS), and the Mini Mental State Examination (MMSE) (*12*).

We used a compartmental model to describe the tracer kinetics in brain and plasma (Fig. 2). The rate constant of [$^{11}$C]NMSP binding to dopamine receptors from the plasma pool and the free and nonspecifically bound radioligand in the brain is $k_3$. For the caudate nucleus, $k_3$ was calculated from the radioactivity in plasma due to unmetabolized tracer and the radioactivity in the caudate nucleus and cerebellum, as described (*8*).

[$^{11}$C]NMSP binds essentially irreversibly to $D_2$ dopamine receptors; there is little or no dissociation from the $D_2$ receptor during the PET imaging time (*13*). The rate of irreversible binding ($k_3$) of [$^{11}$C]NMSP is the product of the bimolecular association rate, $k_{on}$, and the quantity of available receptors ($B'_{max}$). $B'_{max}$ is the difference between the total density of receptors, $B_{max}$, and the density of receptors occupied by endogenous or exogenous competitor. It is assumed that the unlabeled endogenous or exogenous competitor is in equilibrium with the receptor, and that its concentration is not changing during the scan; under these conditions $B'_{max}$ is constant and smaller than $B_{max}$. In the case of irreversible binding of the ligand to the receptor, there is an inverse linear relationship between the concentration of a receptor blocking agent (unlabeled haloperidol in our case) and the rate of ligand binding, described by the equation

$$\frac{1}{k_3} = a_{br}[H]_{br} + b \qquad (1)$$

where $[H]_{br}$ is the concentration of the blocking agent in the brain; $a_{br}$ is the slope, and $b$ is the value of $1/k_3$ when the concentration of the blocking agent is zero. The

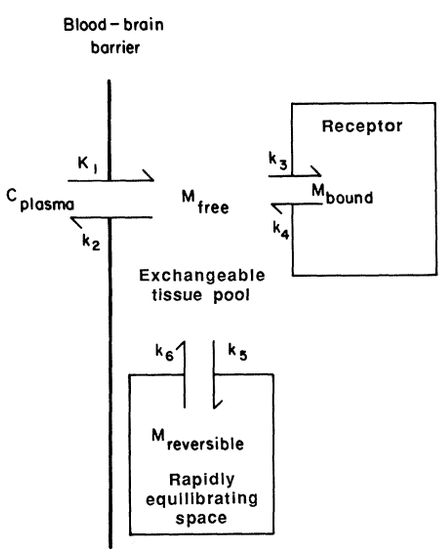


Fig. 2. Schematic representation of the three compartment model. The accumulation of [$^{11}$C]NMSP in the brain occurs in two steps. The ligand first crosses the blood-brain barrier, then binds to the receptors. $C_{plasma}$, concentration of the ligand in arterial plasma; $M_{free}$, quantity of drug in the exchangeable pool of the tissue; $M_{bound}$, quantity of ligand bound to the $D_2$ dopamine receptors; $M_{reversible}$, quantity of drug bound to the secondary or "non-$D_2$" receptors assumed to be in rapid equilibrium with the free ligand in brain; $K_1$, clearance from plasma; $k_2$, rate constant (fractional rate of escape from brain tissue); $k_3$ and $k_4$, the rate constants for the association and dissociation of the ligand to and from the $D_2$ receptors, respectively; $k_5$ and $k_6$, rate constants for the lower affinity or secondary, rapidly reversible binding present in the caudate but not the cerebellum.

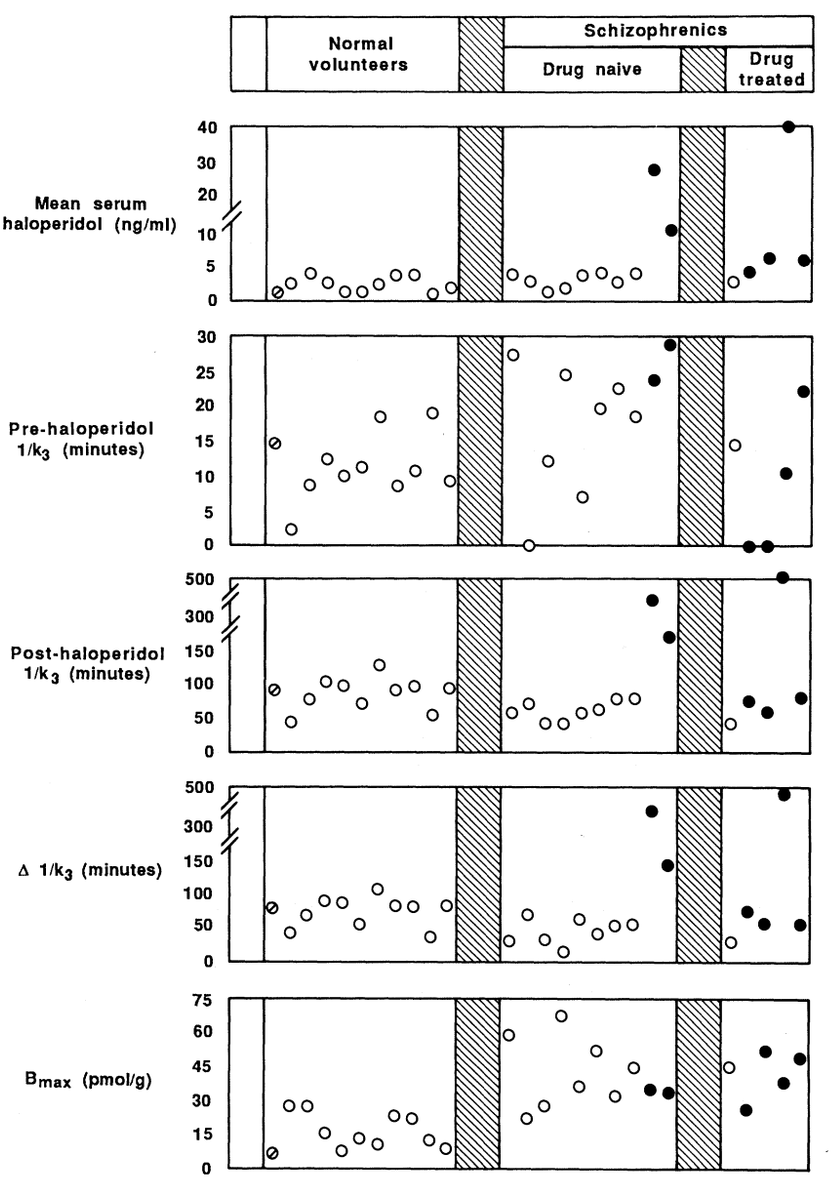


Fig. 3. Individual data for the three groups of subjects. ○, Normal volunteers and schizophrenic subjects that received a single dose of 7.5 mg of haloperidol orally 4 hours before the second PET scan; ⊘, a normal subject who received a single dose of 5 mg of haloperidol rather than 7.5 mg; ●, schizophrenic subjects who received more than a single dose of haloperidol before the second PET scan. These daily doses ranged from 7 to 60 mg for two drug-naive and four drug-treated schizophrenics. In the drug-naive group, all ten subjects had never received neuroleptic medication before the first PET scan. However, the two that had received multiple doses (●) received a daily dose of approximately 30 mg of haloperidol intermittently over a period of 30 days between the first and second PET scans.

slope $a_{br}$ is inversely proportional to the value of the product of the dissociation rate of unlabeled haloperidol in vivo ($k'_{off}$) and $B_{max}$. The abscissal intercept $b$ equals the apparent affinity of the blocking substance (*14*). If the blocking agent in brain is in equilibrium with that in the circulation, it is possible to calculate the brain concentration of the blocking agent from the plasma concentration and the brain-plasma partition coefficient ($\lambda$). $B_{max}$ is calculated from the equation

$$B_{max} = \frac{1}{k'_{off}\, a_{br}} = \frac{\lambda}{k'_{off}\, a_{pl}} \qquad (2)$$

where $a_{pl}$ is the slope of the relationship between $1/k_3$ and haloperidol concentrations measured in plasma (that is, $a_{pl} = a_{br}/\lambda$).

When two measurements of the value of $k_3$ are available for each patient in the unblocked and the blocked state, $B_{max}$ may be calculated from the equation

$$B_{max} = \frac{D_w[H]_{pl}}{\frac{1}{k_{3B}} - \frac{1}{k_{3U}}} \qquad (3)$$

where $D_w$ is the ratio between $\lambda$ and $k'_{off}$ (*15*); $[H]_{pl}$ is the concentration of the blocking agent in plasma; and $k_{3B}$ and $k_{3U}$ are the binding rate constants of the ligand in the blocked and unblocked state, respectively.

The results are summarized for the normal and schizophrenic subjects in Fig. 3 and Table 1. The difference between $1/k_3$ before and after haloperidol administration ($\Delta 1/k_3$) was significantly lower in drug-naive schizophrenic patients than in control subjects. The value of the slope of the line relating $1/k_3$ and serum haloperidol (Fig. 4) reflected the increased receptor blockade with higher plasma levels of haloperidol. The effect of haloperidol on this slope was more pronounced in the control subjects than in the schizophrenic patients.

The density of $D_2$ dopamine receptors was calculated from the ratio of the mean serum haloperidol levels to $\Delta\ 1/k_3$ multiplied by $D_w$. This $B_{max}$ value was substantially higher in both drug-naive and drug-treated schizophrenic patients than in control subjects (Figs. 3 and 5) (*16*). The two normal subjects with the highest $B_{max}$ values were also the youngest (18 years). $B_{max}$ values for our controls averaged 16.6 ± 2.5 (SEM) pmol of [$^{11}$C]NMSP per gram of wet weight tissue, which is in agreement with those of Farde *et al.*, with a different method (*17*).

These results provide evidence that $D_2$ dopamine receptor densities are elevated in chronic schizophrenia, even in drug-naive patients. In a preliminary study of schizophrenic and bipolar affective patients (*18*), we reported the rate of binding of [$^{11}$C]NMSP only in the unblocked state, using a simple ratio of the radioactivity in caudate nucleus divided by that in the cerebellum. No significant differences in our and a similar study (*18*) were found between schizophrenic and control subjects with this index ratio, probably because of the confounding effects of blood flow on this index in subjects with relatively high receptor densities (*19*).

In our study reported here, the number of unoccupied receptors in both the normal subjects and schizophrenic patients was reduced by prior blocking with haloperidol. The reduction of $k_3$ increased the sensitivity of the measured rate of binding to differences of the number of receptors. Apparently, a given level of haloperidol left a greater number of unoccupied $D_2$ dopamine receptors in schizophrenic patients than in control subjects (Fig. 1) (*4*).

The model we used for these calculations requires a number of assumptions, which can be justified on the basis of experimental evidence. We have assumed that haloperidol dissociates from dopamine receptors at the same rate in schizophrenic and control subjects. This seems justified by the fact that the dissociation constant of haloperidol and

Table 1. Average (± SEM) age, serum haloperidol concentration, $1/k_3$ before and after haloperidol treatment, $\Delta\ 1/k_3$, and $B_{max}$ for each of the three groups of subjects.

| Parameter | Units | Subject group | | |
|---|---|---|---|---|
| | | Normal volunteers | Drug-naive schizophrenics | Drug-treated schizophrenics |
| Age | Years | 24.3 ± 2 | 31.2 ± 3.6 | 26.8 ± 2.6 |
| Serum haloperidol* | Ng/ml | 2.6 ± 0.4 | 3.5 ± 0.3 | — |
| $1/k_3$ before haloperidol | Minutes | 11.7 ± 1.4 | 18.5 ± 2.4 | 9.6 ± 4.3 |
| $1/k_3$ after haloperidol* | Minutes | 85.6 ± 7 | 61.2 ± 3.9 | — |
| $\Delta\ 1/k_3$* | Minutes | 74.1 ± 6.6 | 44.6 ± 5.1† | — |
| $B_{max}$ | Pmol/g | 16.6 ± 2.5 | 41.7 ± 4.6† | 43.3 ± 4.7† |

*Average value was calculated only in subjects given a single dose of 7.5 mg of haloperidol before the second PET scan. Serum haloperidol, $1/k_3$ after haloperidol, and $\Delta\ 1/k_3$ depend on the dose of haloperidol given; these values in patients receiving daily doses of haloperidol are different from those in patients given a single dose of haloperidol and therefore cannot be averaged (see Fig. 3). †Significantly different from normal volunteer group value ($P < 0.05$) ($t$ test with Bonferroni correction for multiple inference).

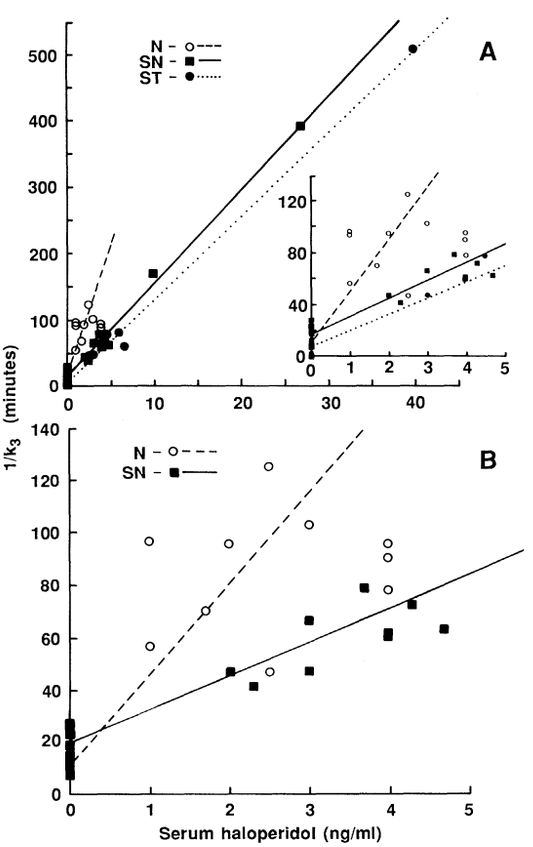


Fig. 4. Comparison between the normal and the schizophrenic subjects of the degree of receptor blockade ($1/k_3$) as a function of serum haloperidol concentration. As indicated in Eqs. 1 and 2, the slope of a plot of $1/k_3$ versus serum haloperidol for each subject provides an average slope that is proportional to the reciprocal of $k'_{off} \cdot B_{max}$. (A) The values of the average slopes (± SEM) for all normal (N), drug-naive (SN), and drug-treated (ST) subjects were 39.0 ± 7.2, 13.3 ± 1.4, and 12.0 ± 1.6 min ng$^{-1}$ ml$^{-1}$, respectively. (Inset) Greater detail near the origin of the graph. The average slopes for the normal and drug-naive groups were significantly different ($P < 0.05$); ($t$ test with Bonferroni correction). (B) The average slopes for the normal (N) and the drug-naive (SN) subjects who received 7.5 mg of haloperidol only were similarly calculated and were 34.9 ± 6.5 and 13.1 ± 1.8 min ng$^{-1}$ ml$^{-1}$, respectively (significantly difference, $P = 0.009$, $t$ test). Because there was only one drug-treated subject who received 7.5 mg of haloperidol this value was not included in the testing procedure.

other neuroleptics is the same in autopsy studies of the brains of schizophrenic and nonschizophrenic subjects (*4, 5*). The model also assumes that the partition coefficient of haloperidol between plasma and brain tissue is the same for patients and normal subjects (*20*). Although it is possible that psychophysiological differences between the control and schizophrenic subjects could affect the pharmacokinetics of labeled or unlabeled neuroleptics, we do not think that this is the case (*21*). We measured the [$^{11}$C]NMSP partition coefficient in each subject to calculate the individual $k_3$ values; we used their average values and in vitro studies to calculate brain haloperidol levels from serum levels (*15, 22*). Average serum haloperidol levels were not significantly different in patients and normal subjects given a single dose of 7.5 mg of haloperidol, in agreement with the reported similar bioavailability of haloperidol in normal volunteers and schizophrenics (*23*).

Possible changes in cerebral blood flow between normals and schizophrenics (*24*) cannot account for the differences in dopamine receptor density because our analysis determines receptor binding rate constants, which are independent of blood flow (*19*).

Enlarged third and lateral ventricles have been reported in x-ray computed tomography (CT) studies of schizophrenics (*25*). Differences in the volume of the caudate nucleus between schizophrenic and control subjects are an important consideration because the increased receptor density we observed could result from either an absolute increase in dopamine receptors compared to normals or a relative loss of cells that don't have dopamine receptors. Differences in caudate size, if present, could also affect quantification of the tracer concentration. Measurement of our x-ray CT scans revealed differences between normals and schizophrenics for third ventricle to brain ratios, but not for estimated caudate size (expressed as either axial dimensions or area), or caudate size corrected for head size (caudate to brain ratio), nor for other CT measures (*26*). Furthermore, neither previously reported changes nor our observations could account for our measured differences in dopamine receptor density (*27*).

Increased dopamine receptor density was not related to the duration of illness. No significant correlations were found between $B_{max}$ estimates and clinical ratings (SANS, BPRS, MMSE), with the exception of the Mini PSE (*28*). The small sample sizes make any interpretations of these findings tenuous.

The finding that $D_2$ dopamine receptors are substantially increased in schizophrenic patients who have never been treated with neuroleptic drugs raises the possibility that dopamine receptors are involved in the schizophrenic disease process itself. Alternatively, the increased $D_2$ receptor number may reflect presynaptic factors such as increased endogenous dopamine levels (*16*). In either case, our findings support the hypothesis that dopamine receptor abnormalities are present in untreated schizophrenic patients. Nevertheless, it is important to measure dopamine receptor densities in patients with nonschizophrenic psychotic disturbances such as affective disorders (*29*) to determine whether the dopamine receptor abnormality is characteristic of schizophrenia. Until such studies in other psychiatric disorders are performed, it would be premature to conclude that dopamine receptor abnormalities are specific for schizophrenia (*30*).

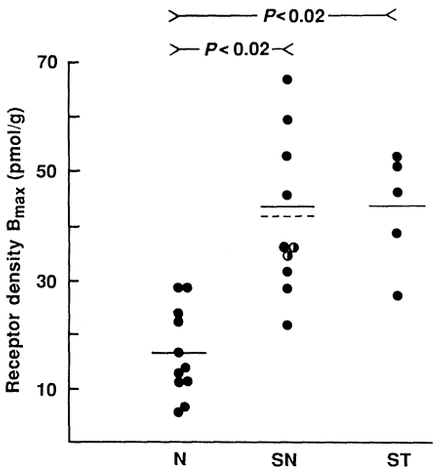


Fig. 5. $D_2$ dopamine receptor density ($B_{max}$) in the caudate nucleus in normal volunteers (N) and drug-naive (SN) and drug-treated (ST) schizophrenics. The solid horizontal lines are the mean values in each group. For the drug-naive group this line is the value for the eight subjects who had only a single 7.5-mg dose of haloperidol before their second PET scan [43.3 ± 5.7 pmol/g (SEM)]. The dotted line below it is the mean of all ten subjects, including the two who received more than a single dose of haloperidol before their second PET scan (◑). The average receptor density of this group was 41.7 ± 4.6 pmol/g. Mean receptor densities for the normal volunteers and the drug-treated group were 16.6 ± 2.5 and 43.3 ± 4.7 pmol/g, respectively. There was a significant difference between either the eight or ten drug-naive or the drug-treated schizophrenics and the normal subjects ($t$ test with Bonferroni correction for multiple inference).

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8. D. F. Wong *et al.*, *ibid.* **226**, 1393 (1984); D. F. Wong *et al.*, *J. Center. Blood Flow Metab.* **6**, 147 (1986). Each PET scan began with the injection of 15 to 20 mCi of [$^{11}$C]NMSP into an antecubital vein over 10 to 20 seconds. The [$^{11}$C]NMSP was labeled [H. D. Burns *et al.*, *J. Nucl. Med.* **25**, 1222 (1984); R. F. Dannals, H. T. Ravert, A. L. Wilson, H. N. Wagner, Jr., *Int. J. Appl. Radiat. Isot.* **36**, 433 (1986)] to a specific activity averaging 1000 to 2000 Ci/mmol at the time of synthesis. The injected mass averaged 0.1 ± 0.01 μg of [$^{11}$C]NMSP per kilogram of body weight, as determined by high-performance liquid chromatography (HPLC). There was no significant difference between the specific activities of [$^{11}$C]NMSP in the normal or patient groups either before or after haloperidol treatment. The emitted radioactivity was recorded with the NeuroECAT PET scanner (C.T.I.) in the high-resolution mode. Radioactivity was simultaneously detected in the caudate nucleus and cerebellum for 80 to 90 minutes after the injection of the tracer. The temporal sequence and scan length was as follows: five scans of 2 minutes, five scans of 5 minutes, one scan of 15 minutes, and one scan of 30 minutes. Blood samples were obtained from the dorsal vein of the hand contralateral to the injection site; the hand was heated to 44°C to "arterialize" the venous samples. In some cases radial arterial samples were obtained simultaneously. The temporal sequence of blood sampling after the injection of [$^{11}$C]NMSP was: Four to six samples the first minute, three to six the second minute, two the third minute, then every minute up to the tenth minute, then every two minutes up to the twentieth minute, then at 25, 30, 45, 60, 75, and 90 minutes. Blood samples were then centrifuged and plasma samples counted in a gamma scintillation spectrometer.

Our procedure usually employed arterialized venous sampling for the input function because our method is based on the calculation of integrals and net rates of influx. Hence, uncertainty about the early part of the blood curve of the tracer is not likely to influence the estimate of $k_3$. In five cases of comparison between the arterial and arterial-venous measurements, the average difference of $1/k_3$ was 3.7 ± 2% (SD) and of $B_{max}$ was 3.9 ± 2%.

Our estimates of $k_3$ are based on a slope-intercept method that asymptotically estimates net rate of binding, the distribution volumes of the ligand in brain, and the volume of distribution of any rapidly equilibrating binding sites ($M_r$). Because the model involves six transfer coefficients, such asymptotic procedures are used. The data are obtained with a sampling time resolution that may not be sufficient to estimate transfer coefficients by a traditional numerical solution and parameter estimation of the differential equations.

The use of this model requires a number of assumptions to obtain accurate values of absolute receptor density. These include good estimates of $k'_{off}$ and the partition coefficient for haloperidol. An additional assumption is that $k_{on}$ for [$^{11}$C]NMSP and for haloperidol are not significantly different,

and that the tracer dose does not result in a significant decrease in the number of available receptors. It is possible that, with improvements in the measurements of these values, the estimate of $B_{max}$ might change. Nevertheless, our conclusions depend only on the relative differences in receptor density between normals and patients. This is because we assume that values such as $k'_{off}$ are not different in normals and schizophrenics, and that the principal differences in $B_{max}$ are due to differences in the rate constant ($k_3$) measured by PET in the presence of various haloperidol levels. This method utilizes a single haloperidol partition coefficient and individual serum haloperidol levels and is a slight modification of the model and analysis algorithm previously reported [D. F. Wong, A. Gjedde, H. N. Wagner, Jr., *J. Cereb. Blood Flow Metab.* **6**, 137 (1986); D. F. Wong *et al.*, *ibid.*, p. 147].

9. Each subject had three to five plasma samples drawn during the second PET scan. The samples were assayed for haloperidol by Gas Chromatography at the National Psychopharmacology Lab, Nashville, Tennessee. The laboratory assay sensitivity is 0.2 ng of haloperidol per milliliter of plasma. There is a coefficient of variation for plasma of 1 to 2.1% over a range of 5 to 25 ng per milliliter of plasma and for red cells of 1.2 to 5.9% over a range of 2.5 to 10 ng per milliliter of red cells. The assay separates and excludes the inactive metabolite, reduced haloperidol [D. L. Garver, J. Hirschowitz, G. A. Glicksteen, D. R. Kanter, M. L. Mavroidis, *J. Clin. Psychopharmacol.* **4**, 133 (1984)].
10. The DSM III schizophrenic subclassification for the drug-naive subjects was seven undifferentiated and three paranoid patients. For the drug-treated subjects, there were two undifferentiated, one paranoid, and two disorganized. The subjects were regarded as a sample of drug-naive and drug-treated schizophrenic patients meeting DSM III criteria.
11. Of particular interest in the drug-naive subjects is the relatively long duration of illness, 5 ± 3 years (SD), before receiving neuroleptic treatment. Those patients with the longest duration of illness did receive other medical or psychiatric interventions, in the form of psychotherapy (one patient received 10 years of psychoanalysis) or prolonged medical assessments for delusions concerning physical health. Most of these patients expressed long-standing reluctance to accept either psychotropic medications or psychiatric diagnoses and therefore might be an atypical group. However their symptomatology as characterized by the BPRS and present state examination was typical of chronic schizophrenic illness.
12. J. E. Overall and D. R. Gorham, *Psychol. Rep.* **10**, 799 (1962); J. K. Wing *et al.*, *Measurements and Classification of Psychiatric Symptoms* (Cambridge Univ. Press, Cambridge, 1974); M. F. Folstein *et al.*, *J. Psychiatr. Res.* **12**, 189 (1975); N. Andreasen and S. Olsen, *Arch Gen. Psychiatry* **39**, 789 (1982).
13. [$^{11}$C]NMSP is not highly lipophilic ($K_1$ averaged approximately 0.15 ml g$^{-1}$ min$^{-1}$ in our subjects, corresponding to an initial extraction fraction of no more than 30%) but must enter neurons with the same ease that it crosses endothelium cells. Thus, the ligand has access to internalized as well as membrane bound receptors [D. C. Chugani, R. F. Ackermann, M. E. Phelps, *Soc. Neurosci. Abst.* **12**, 417 (1986)] that cannot be distinguished by our model. In addition, some of the receptors measured may not be functional.
14. The abscissal intercept $b$ is the product of the apparent affinity ($K'_1$) and the volume of distribution of the tracer ($V_d$). Both $B_{max}$ and $K'_1$ can be determined graphically for individuals undergoing multiple PET scans with haloperidol blocking doses or from Eq. 3, and from Eqs. 10 and 11 in D. F. Wong *et al.* [D. F. Wong *et al.*, *J. Cereb. Blood Flow Metab.* **6**, 147 (1986)].
15. The "drug weight" ($D_w$) value [494 (pmol-minutes/g)/(μg/ml)] was determined as the estimated partition coefficient of haloperidol between brain and plasma (2.6 ml/g) divided by the product of the molecular weight of haloperidol (375.88 g/mol) and the in vivo $k'_{off}$ of haloperidol (0.014/minute). The brain-plasma partition coefficient for haloperidol was determined both from average (SD) values of the $K_1/k_2$ ratios of [$^{11}$C]NMSP for normals [2.1 ± 0.9 (SD)] and schizophrenic subjects (1.9 ± 1) (nonsignificant differences for all groups) and by in vitro experiments. The latter studies consisted of incubating [$^3$H]haloperidol in human plasma (containing plasma proteins) with minced "prisms" (0.5 mm) of rat cerebellum (30 mg/ml) (which had been incubated with 100 n*M* d-SKF 10047 to block cerebellar sigma receptors) at 37°C for 60 minutes, a time at which tissue radioactivity was maximal. The partition coefficient was then calculated both by monitoring the loss of radioactivity from the medium, and from the content of radioactivity entering the brain tissue, yielding partition coefficients of 3.1 and 2.8, respectively. For this study we used 2.6 as an estimate of the partition coefficient of haloperidol. The partition of haloperidol is affected by the presence of plasma proteins, as we have demonstrated in vitro by the addition of plasma to a radioactive saline media containing the tissue prisms. Thus in vivo it is also likely that the partition depends on plasma elements, including proteins. But the effective free fraction of haloperidol can be greater in vivo than in vitro, so it is not possible to easily predict the change in the partition coefficient of haloperidol from in vitro experiments alone.
16. This method of analysis does not permit determination of affinity [dissociation constant ($K_D$)] of [$^{11}$C]NMSP but it allows the calculation of the apparant inhibitory constant of haloperidol for the $D_2$ receptor. Because of the proximity of the value of $1/k_3$ to the origin of the graph in the unblocked state, estimates of $K'_1$ from the abscissal intercept are considerably less certain than estimates of $B_{max}$ from the slope of the relation of $1/k_3$ to haloperidol. Nevertheless this apparent inhibition constant $K'_1$ averaged for the normal, drug-naive, and drug-treated patients 2.1 ± 1 (SD), 11.9 ± 8, and 5.7 ± 6 n*M*, respectively. Unlike affinities determined under in vitro conditions these are measured in the presence of endogenous neurotransmitters. Even though [$^{11}$C]NMSP has a very high affinity for the $D_2$ receptors compared to dopamine, this finding could be interpreted as preliminary evidence for an intrasynaptic dopamine excess, especially in the drug-naive subjects compared to normals. This may also suggest that prior treatment is associated with lower endogenous neurotransmitter levels, closer to normal values.

    An alternative explanation is possible if endogenous ligand is not a major factor in the changes of $K'_1$. In this case true affinity alterations in the $D_2$ receptor in schizophrenics may occur that might decrease the differences in the receptor densities, but imply dramatic affinity differences between schizophrenic patients and normal subjects. Hence, our primary hypothesis that dopamine receptors are abnormal in drug-naive schizophrenics would not change.
17. Farde and colleagues [L. Farde, H. Hall, E. Ehrin, G. Sedvall, *Science* **231**, 258 (1986)] used PET, [$^{11}$C]raclopride, and an equilibrium displacement approach to determine the $B_{max}$ values in normal volunteers (the average $B_{max}$ value was 14.4 pmol per gram of wet weight tissue for similar aged controls. Values for the schizophrenic subjects were not published.
18. D. F. Wong *et al.*, *Psychopharmacol. Bull.* **21**, 595 (1985); S. Herold *et al.*, *J. Cereb. Blood Flow Metab.* **5** (suppl. 1), S191, 1985.
19. The ratio between radioactivity in the caudate nucleus (binding region) and the cerebellum (a nonbinding region) (Ca/Cb ratio) has been used routinely as an index of neuroreceptor-radioligand interaction (*7*). For a ligand such as [$^{11}$C]NMSP that continuously accumulates in the caudate nucleus without coming to an equilibrium between bound and unbound ligand, the Ca/Cb ratio will continue to increase as a function of time. The rate of increase of this ratio (slope) has therefore been used as an index of the rate of binding of the ligand. However the slope of the Ca/Cb ratio as a function of time also depends on the blood flow rate to the caudate nucleus and, if binding is very rapid, as in many of the schizophrenic patients, it is dominated by and reflects blood flow. Because receptors were not blocked with haloperidol in the original study, it is not surprising that the indices did not differ between patients and controls. The independent determination of the binding constant $k_3$ eliminates the problem of the blood flow dependence of the value of $K_1$ [D. F. Wong, A. Gjedde, H. N. Wagner, Jr., *J. Cereb. Blood Flow Metab.* **6**, 137 (1986); D. F. Wong *et al.*, *ibid.*, p. 147]. Only in the case of negligible binding compared to efflux ($k_3 << k_2$) is the slope actually proportional to the rate of binding [D. F. Wong *et al.*, *Science* **232**, 1269 (1986)]. If the rate of binding is very rapid (that is, when $k_2$ is on the order of or less than $k_3$, as suspected in young normal or schizophrenic subjects with higher receptor densities), the slope loses its dependence on the value of $k_3$ and depends more on blood flow.
20. The validity of this argument stems from the hypothesis that the solubility of haloperidol in brain is unlikely to differ in patients and normal subjects because it depends principally upon the characteristics of the parenchyma and not upon blood-brain barrier, receptor, or neurotransmitter differences.
21. There was no significant difference in the pulse, blood pressure, or other indicators of physiological state among normal and schizophrenic subjects. Although pathophysiological differences may result in changes in blood flow, these effects are not likely to affect our results because the analysis yields a value of $k_3$ for [$^{11}$C]NMSP that is independent of the magnitude of blood flow.
22. The activity of [$^{11}$C]NMSP is measured for each brain region over the entire PET imaging period. Because the binding of [$^{11}$C]NMSP during this time is not in equilibrium between free and receptor bound pools, it is necessary to individually account for the pharmokinetics of [$^{11}$C]NMSP in each subject. Thus if differences occur between patients and controls in the pharmokinetics of [$^{11}$C]NMSP, these are directly accounted for in the model. In the case of haloperidol, however, it is assumed that it reaches an equilibrium between bound and free receptor pools during the 4 hours before the PET scan; therefore only an average partition coefficient is needed for each person.
23. J. R. Magliozzi and L. E. Hollister. *J. Clin. Psychiatry* **46**, 20 (1985).
24. Most studies examining cerebral blood flow in schizophrenic patients have shown no significant changes in whole-brain blood flow. Among reported but controversial alterations are a reduction of cerebral blood flow in frontal brain regions and abnormalities in cerebral laterality [D. R. Weinberger, K. F. Berman, R. F. Zec, *Arch. Gen. Psychiatry* **43**, 114 (1986); G. Sheppard *et al.*, *Lancet* **1983-II**, 1448 (1983).
25. E. C. Johnstone, T. J. Crow, C. D. Frith, J. Husband, L. Kreel, *Lancet* **1976-II**, 924, (1976); G. D. Pearlson and A. E. Veroff, *ibid.*, **1981-II**, 470 (1981); J. Boronow *et al.*, *Arch. Gen. Psychiatry* **42**, 266 (1985); K. E. Goetz and D. P. Van Kammen, *J. Nerv. Ment. Dis.* **174**, 31 (1986).
26. X-ray CT scans were performed as described (*8*). The following parameters were measured by individuals unaware of the treatment conditions: total brain area, third ventricle to brain ratio, linear ventricular index (frontal horns), caudate size (major and minor axis lengths), estimated caudate area, and caudate to brain ratio. A CT slice passing through the cavities of the lateral ventricles was not available. Lateral ventricle to brain ratio therefore could not be assessed. A statistically significant difference was observed between drug-naive schizophrenics and normal subjects only for the third ventricle–brain ratio ($P < 0.01$, $t$ test, Bonferroni correction) compared to normal controls, but not for the other CT parameters. Third ventricle to brain ratios (± SD) for drug-naive schizophrenics, drug-treated schizophrenics, and normal subjects were 0.82 ± 0.19, 0.72 ± 0.56, and 0.52 ± 0.21, respectively.
27. The observed radioactivity from the caudate nucleus is a function of its size, especially for small objects that are close to the resolution of the PET scanner (8 mm in plane, 15 mm axial). The true activity of these structures will be underestimated because of a partial volume effect. To estimate these effects on our measurements, we examined caudate size from the x-ray CT scan in the plane of the PET scan and computed the required correction factor (recovery coefficient) by assuming the caudate head to be a sphere. Using phantom studies with spheres, we know the true corrections for partial volume loss [D. F. Wong *et al.*, *J. Nucl. Med.* **25**, 105 (1984)]. There was no significant difference between normal, drug-naive, and drug-treated subjects in either caudate area [2.1 ± 0.31, 2.0 ± 0.33, and 2.3 ± 0.43 cm$^2$, respectively] nor recovery coefficient [0.55 ± 0.11, 0.47 ± 0.10, and 0.61 ± 0.15, respectively]. When expressed as a fraction of whole brain area, there was also no significant difference in caudate sizes. The partial volume effect, if present at all, would cause an underestimation of receptor density in the drug-naive subjects.
28. There was an inverse correlation between schizophrenic symptoms in the drug-naive subjects, as measured by the Mini PSE, and receptor density $B_{max}$. The correlation coefficient for these jointly bivariate variables in drug-naive subjects was $r =$

$-0.6$ ($P < 0.05$). The Mini PSE, however, rates only reported positive symptoms. Many of our patients were noted to be suspicious and guarded, probably resulting in an artificially low symptom score. Many patients had more severe symptoms on following examinations.

29. In a preliminary study of two patients with bipolar depression (44 and 52 years of age and one patient with unipolar depression (39 years of age), who were severely depressed at the time of PET scanning, the $B_{max}$ values were 5, 15, and 12.5 pmoles/g respectively.

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# CD8$^+$ Lymphocytes Can Control HIV Infection in Vitro by Suppressing Virus Replication

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**Lymphocytes bearing the CD8 marker were shown to suppress replication of human immunodeficiency virus (HIV) in peripheral blood mononuclear cells. The effect was dose-dependent and most apparent with autologous lymphocytes; it did not appear to be mediated by a cytotoxic response. This suppression of HIV replication could be demonstrated by the addition of CD8$^+$ cells at the initiation of virus production as well as after several weeks of virus replication by cultured cells. The observations suggest a potential approach to therapy in which autologous CD8 lymphocytes could be administered to individuals to inhibit HIV replication and perhaps progression of disease.**

THE ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) is caused by a newly recognized human retrovirus that is now termed human immunodeficiency virus (HIV) (*1*). This virus can be recovered from cultured peripheral blood mononuclear cells (PMC) of individuals with AIDS, AIDS-related conditions (ARC), and many asymptomatic individuals in the known risk groups (*2*). Studies in our laboratory have indicated that cultured PMC from 50% of seropositive healthy individuals do not yield infectious virus (*3*). Moreover, we have studied some individuals whose PMC in cultures have initially released virus and then ceased to yield any infectious virus for more than 1 year (*4*). These individuals remain clinically healthy and show an improvement in their immune status. Their clinical state suggests a control of the virus infection.

Cellular immune responses provide a major mechanism for reducing the growth of virus-infected cells as well as tumors (*5*). We therefore examined whether the lack of production of infectious HIV by the PMC of some individuals was due to selected cellular immune responses. We found that the CD8 (OKT8/Leu-2) subset of T lymphocytes (*6*) suppresses HIV replication in PMC.

For these studies, we removed the CD8$^+$ cells from the PMC of HIV antibody–positive individuals by the panning method of Wysocki and Sato (*7*) (see legend to Table 1). The cells remaining in the CD8-depleted fraction were then cultured in the presence of phytohemagglutinin (PHA) and interleukin-2 (IL-2) (*2, 3*). The removed CD8$^+$ cells were cultured in a similar manner. The supernatants of all cultures were assayed at 3- to 4-day intervals for the presence of HIV (*2, 3*).

In several repeated experiments, cultured unseparated PMC obtained from three healthy, HIV antibody–positive homosexual men (subjects 1, 2, and 3) did not yield infectious HIV (*8*). In contrast, when cultures of PMC from these individuals were depleted of CD8$^+$ cells, substantial levels of reverse transcriptase (RT) activity were detected in the supernatants (Table 1). That these supernatants contained infectious virus was demonstrated by their ability to infect cultured PMC from virus-negative donors, in which they induced RT activity and HIV antigen production (*3*). PMC from subjects 2 and 3 did not release virus after depletion of cells expressing CD16 (Leu-11), a marker associated with natural killer (NK) cells (*9*). However, HIV was detected in the CD16-depleted fraction of PMC from subject 1, a seropositive Asian male. Like other Asian individuals (*10*), he may have a large proportion of CD16$^+$ lymphocytes that co-express the CD8 marker.

High levels of virus-associated RT activity were also detected in CD8-depleted PMC from subject 4, an asymptomatic individual who has had Kaposi's sarcoma for over 4 years. However, low but detectable levels of HIV were also consistently detected in cultures of his unseparated PMC (Table 1). This finding suggests that his CD8$^+$ cells have a reduced capacity to control HIV replication. Virus was not recovered from the cultured CD8$^+$ cells of any of these four individuals; this observation confirms the lack of replication of HIV in this subset of lymphocytes (*11*).

In examining further the role of CD8$^+$ cells in suppressing HIV replication, we performed additional studies on subject 3, who agreed to be tested on several occasions. First, we added his separated CD8$^+$ cells to his autologous CD8-depleted PMC prior to the initiation of culture (Fig. 1). No RT activity was detected in the fluid of the reconstituted culture. We found a clear dose-response relation between the number

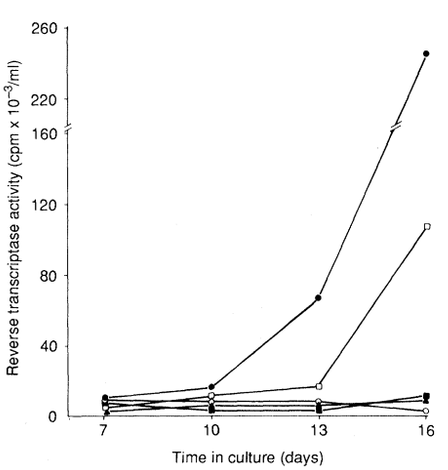


Fig. 1. Reconstitution of CD8-depleted PMC cultures with autologous CD8$^+$ lymphocytes prevents HIV replication. A representative experiment is shown. PMC from subject 3 were separated into CD8$^-$ and CD8$^+$ fractions by the panning method and cultures were established as described in the legend to Table 1; $4 \times 10^6$ CD8$^-$ PMC were cultured alone (●) or with $0.375 \times 10^6$ (□), $0.75 \times 10^6$ (■), or $1.5 \times 10^6$ (▲) autologous CD8$^+$ lymphocytes that were added prior to the initiation of culture. A control culture of $4 \times 10^6$ CD8$^+$ positive cells was also established (○). All culture supernatants were monitored for HIV-associated RT activity at 3- to 4-day intervals, and the presence of HIV antigen in cultured cells was confirmed by an indirect immunofluorescence assay (see legend to Table 1).

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