

from the complex will be small and will lead to large statistical (radioactivity counting) errors and that the signal from the unbound or nonspecifically bound NMSP in the brain may interfere with the signal from the complex. The $1/k_3$ value derived in the presence of haloperidol is the primary determinant of the slope [figure 4 of (3)] used to quantify $B_{\max}$. Values for $1/k_3$ determined in the absence of haloperidol may be inaccurate because, for high levels of available receptor, the kinetics of [^{11}C]NMSP accumulation in the caudate nucleus primarily reflect blood-brain transport and are relatively insensitive to the second-order binding step k_{on} , so there may be significant errors associated with the numerical extraction of k_3 from the observed data. Values for $1/k_3$ derived in the absence of haloperidol would be the primary determinant of the y-intercept [figure 4 of (3)]. In the absence of additional information about the analysis of the original experimental data or a sensitivity analysis based on computer simulation studies, it is not clear what the magnitude of these errors might be.

5. The y-intercept and $1/k_3$ before haloperidol treatment will be identical, since $1/k_3$ before haloperidol treatment is one of the two points used in the $1/k_3$ versus haloperidol plot.
6. Since K'_1 , equal to $k'_{\text{off}}/k'_{\text{on}}$ and determined from the x-intercept of the $1/k_3$ versus haloperidol plot, is different for normal individuals and for schizophrenics (1), k'_{off} or k'_{on} , or both, must be different for normal individuals and for schizophrenics. If k'_{off} differs, then in the implementation of the slope method a single assumed constant value cannot be used for both normal individuals and for schizophrenics. If k'_{on} differs, one would need to assume that k_{on} also differs in such a way that $k_{\text{on}}(\text{normal})/k_{\text{on}}(\text{schizophrenic})$ is identical to $k'_{\text{on}}(\text{normal})/k'_{\text{on}}(\text{schizophrenic})$.
7. D. F. Wong *et al.*, *Science* **232**, 1270 (1986).
8. D. F. Wong *et al.*, *J. Nucl. Med.* **27**, 1074 (1986).
9. P. Seeman, *Pharmacol. Rev.* **32**, 229 (1981); A. A. Hancock and C. L. Marsh, *Mol. Pharmacol.* **26**, 439 (1984).
10. In addition to the direct result of haloperidol and dopamine competition in [^{11}C]NMSP binding to the D_2 receptor, an analysis of the effects of endogenous dopamine and added haloperidol must take into account the indirect result of the influence of haloperidol on the turnover of dopamine, on occupancy, functioning, and regulation of the presynaptic dopamine autoreceptor and the postsynaptic D_1 , D_2 , and D_3 dopamine sites, and on the levels of [^{11}C]NMSP and dopamine in the synaptic cleft. For example, D. C. Chugani *et al.* [*J. Nucl. Med.* **28**, 612 (1987)] have shown that endogenous dopamine can increase in vivo ^3H -spiperone binding by stimulation of endocytotic trapping.

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Response: We appreciate the comments of Zeeberg *et al.*, which give us an opportunity to amplify our conclusions. On the basis of simple receptor kinetic theory, we predicted that the reciprocal of the binding coefficient ($1/k_3$) must be a linear function of the inhibitor (haloperidol) concentration (I). The plot of $1/k_3$ versus haloperidol concentration is essentially a Woolf plot (2) in which the slope equals the value of $1/[k'_{\text{off}} B_{\max}]$, where k'_{off} is the in vivo rate of dissociation of haloperidol from the receptor sites, corrected for the ratio between the k_{on} values of (3-N-[^{11}C]methyl)spiperone ([^{11}C]NMSP) and haloperidol. Zeeberg *et al.* assert that it may be equally valid to calculate the value of $B_{\max}$ as the ratio between k_3 and k_{on} in the absence of any inhibitor and subsequently use the published values of the ordinate intercept to

suggest a contradiction in our conclusion that $B_{\max}$ is higher in drug-naïve schizophrenic patients. The data on the ordinate intercept and affinity were cautiously presented in our report so that future studies might shed light on issues such as possible elevated neurotransmitter levels in schizophrenics, while the principal point of the report concerned receptor densities. We believe the empirical differences and the theoretical arguments that lead to the assertions of Zeeberg *et al.* are not robust. In fact, the observed values of the ordinate intercept in patients and controls are quite compatible with our original thesis.

The argument extended by Zeeberg *et al.* is drawn solely from comparisons between patient and control groups for the ordinate intercept values. However, as we stated (3), these differences were not statistically significant. Basing the calculation of $B_{\max}$ on the assumed value of k_{on} , rather than on the assumed value of k'_{off} , did not work in our experience for several reasons. First, we did not know the in vivo value of k_{on} for [^{11}C]NMSP, while an estimate of k'_{off} could be obtained from the literature. Second, estimates of k_3 in the absence of inhibition are more uncertain than in the presence of inhibitor because binding to unblocked receptor sites is sometimes so intense that delivery of tracer from the circulation to tissue may affect the accuracy of the binding estimates. Measurement of cerebral blood flow will not facilitate the calculation of the binding rate in this situation, but will merely confirm that binding has little influence on the rate of tracer accumulation. Third, in our case with the use of Woolf plot, the theoretical and experimental accuracy of $B_{\max}$, determined as the reciprocal value of a slope, has a much lower relative variance than the K_D estimated from the ordinate intercept (2, 3). Fourth, the solution of the equation for $B_{\max}$ incorporates k_3 both in the presence and in the absence of haloperidol. The $1/k_3$ averages near the origin of the graph have less influence on the calculated $B_{\max}$ values for relatively sizable haloperidol concentrations because the k_3 value in the presence of haloperidol dominates the calculation. In our data $1/k_3$ observed in the absence of haloperidol is on average only 25% (0 to 60%) of the value of $1/k_3$ obtained in its presence.

Even if the ordinate intercept values were significantly different between patients and controls, they would merely imply a greater increase of the observed k_{on} for control subjects than for drug-naïve schizophrenic patients. An increased total number of dopamine receptors in schizophrenics may accompany a decreased rate of association, which is reflected in our report of a higher

K'_1 value for haloperidol. Reduced affinity is a common consequence of up-regulation of receptors, perhaps due to impaired access to the receptor sites in vivo or large increases in endogenous neurotransmitter competition. The ratio between the rates of net binding of methylspiperone in the haloperidol-blocked and unblocked cases is a model-independent estimate of the in vivo affinity of haloperidol.

An isolated increase of this value of K'_1 in drug-naïve schizophrenics would be difficult to explain, save by a decrease of the in vivo value of k_{on} . In fact, we stated that an increase in K'_1 (and logically k_{on}) could be predicted and be consistent with our analysis and findings.

Zeeberg *et al.* argue that our previous reports indicate a lack of endogenous competition with NMSP binding to receptors. The studies of cocaine administration to young subjects used a different modeling approach, the so-called caudate/cerebellar ratio method, which may reflect both flow and receptor binding (reference 19 of our report). Given our current kinetic approach, the lack of change in the caudate/cerebellar ratio in the presence of intravenous cocaine does not exclude a reduction in the rate of binding of [^{11}C]NMSP due to endogenous competition (4).

An initial assumption of setting the endogenous effect to zero ($B_{\max} = B'_{\max}$, in the unblocked case) would be reflected in an increase in k_{on} if such competition should occur. Alternatively, an increase of k_{off} to explain the increase K'_1 would counter the argument advanced by Zeeberg *et al.* In this case we are left with the conclusion drawn in our original report, that is, that receptor numbers are elevated in drug-naïve schizophrenics. We showed an elevation in drug-naïve as well as in previously treated patients, in whom increased receptors have frequently been confirmed post-mortem. The criticism of Zeeberg *et al.* would equally affect this latter group of patients, if valid.

Zeeberg *et al.* also make comments regarding computations which may benefit from our clarification and response. They compare $1/k_3$ averages in the haloperidol-blocked cases. However, this comparison has meaning only when the haloperidol levels in blood are the same in normals and schizophrenics. When Zeeberg *et al.* averaged the $1/k_3$ values from figure 3, they apparently included values obtained at different haloperidol levels and thus did not correctly represent the "slope" differences. All haloperidol levels and corresponding $1/k_3$ values are shown in figure 3; $B_{\max}$ differences were dependent on the "slopes," but not on either $1/k_3$ or haloperidol alone. Zeeberg *et al.* also find it surprising to

observe a 2.5-fold increase in the $B_{\max}$ of schizophrenics, given the presence of the relatively small changes in $1/k_3$ in the presence of haloperidol. In fact it is the within-subject differences between unblocked and blocked values of $1/k_3$ at various haloperidol levels that provide the twofold density difference between patients and controls.

Estimation of binding at several inhibitor concentrations improves the certainty of the $B_{\max}$ value by confirming the prediction of linearity between the value of $1/k_3$ and the inhibitor concentration. We found it clinically feasible only to do two studies per drug-naïve schizophrenic subject. We have recently carried out more than two studies per patient which demonstrate linearity and thus can provide an important validation of our methodology. We believe the slope method is the most reasonable and accurate measure of $B_{\max}$ at present.

While future studies may demonstrate factors as the internalization of ligand-receptor complexes, and a more sophisticated accounting of possible endogenous compe-

tion, the data presented can be best explained with receptor alterations in drug-naïve schizophrenics. On the basis of the current data, observed or actual receptor affinity changes cannot be ruled out, but the most plausible interpretation is an increase of the density of receptor sites in schizophrenic patients.

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REFERENCES AND NOTES

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2. N. A. Cressie and D. D. Keightley, *Biometrics* **37**, 235 (1981).
3. In the theoretical case of two haloperidol concentrations (one being zero) and low haloperidol measurement error, first approximation of the relative variance of the slope estimate is smaller than that of the ordinate intercept estimate when mean haloperidol levels exceed the $\sqrt{2}$ (intercept/slope ratio). The haloperidol level exceeded this requirement in the majority of our cases. For our empirical data, values of $1/k_3$ in the unblocked cases had higher relative variance in the intercept than in the slope at a given haloperidol concentration. For example, in patients and controls with similar haloperidol concentrations, the relative variances for ordinate intercepts and slopes, respectively, were 0.022, 0.010 for normal controls and 0.21, 0.057 for drug-naïve patients.
4. D. F. Wong *et al.*, *J. Nucl. Med.* **27**, 1074 (1986).
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