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/k3 value derived in the presence of haloperidol is the primary determinant of the slope [figure 4 of (3)] used to quantify Bmax. Values for 1/k3 determined in the absence of haloperidol may be inaccurate because, for high levels of available receptor, the kinetics of [1C]NMSP accumulation in the caudate nucleus primarily reflect blood-brain transport and are relatively insensitive to the second-order binding step koff so there may be significant errors associated with the numerical extraction of k3 from the observed data. Values for 1/k3 derived in the absence of haloperidol would be the primary determinant of the reciprocal of the slope in vivo [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 simulations study, it is not clear what the magnitude of these errors might be.

5. The y-intercept and 1/k3 before haloperidol treatment will be identical, since 1/k3 before haloperidol treatment is one of the two points used in the 1/k3 versus haloperidol plot.

6. Since K1, equal to koff/k. Kon and determined from the x-intercept of the 1/k3 versus haloperidol plot, is different for normal individuals and for schizophrenics (1), koff or k. Kon or both, must be different for normal individuals and for schizophrenics. If koff differs, then in the implementation of the method a single assumed constant value cannot be used for both normal individuals and for schizophrenics. If k. Kon differs, one would need to assume that the incorporation of [1C]NMSP, while an estimate of koff could be obtained from the literature. Second, estimates of k3 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 Wolff plot, the theoretical and experimental accuracy of Bmax, determined as the reciprocal value of the slope, has a much lower relative variance than the KD estimated from the ordinate intercept (2, 3). Fourth, the solution of the equation for Bmax incorporates k3 both in the presence and in the absence of haloperidol. The 1/k3 averages near the origin of the graph have less influence on the calculated Bmax values for relatively sizable haloperidol concentrations because the k3 value in the presence of haloperidol dominates the calculation. In our data 1/k3 observed in the absence of haloperidol is on average only 25% (0 to 60%) of the value of 1/k3 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 koff for control subjects than for drug-naive 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 K1' 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 K1' in drug-naive schizophrenics would be difficult to explain, save by a decrease of the in vivo value of koff. In fact, we stated that an increase in K1' (and logically koff) 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 [1C]NMSP due to endogenous competition (4).

An initial assumption of setting the endogenous effect to zero (Bmax = B'max, in the unblocked case) would be reflected in an increase in koff if such competition should occur. Alternatively, an increase of koff to explain the increase K1' would count 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-naive schizophrenics. We showed an elevation in drug-naive 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/k3 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/k3 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/k3 values are shown in figure 3; Bmax differences were dependent on the "slopes," but not on either 1/k3 or haloperidol alone. Zeeberg et al. also find it surprising to

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/k3) must be a linear function of the inhibitor (haloperidol) concentration (1). The plot of 1/k3 versus haloperidol concentration is essentially a Wolff plot (2) in which the slope equals the value of 1/(koff Bmax), where koff is the in vivo rate of dissociation of haloperidol from the receptor sites, corrected for the ratio between the kon values of (3-N-[1C]methyl)spiperone ([1C]NMSP) and haloperidol. Zeeberg et al. assert that it may be equally valid to calculate the value of Bmax as the ratio between k3 and kon in the absence of any inhibitor and subsequently use the published values of the ordinate intercept to
observe a 2.5-fold increase in the $B_{\text{max}}$ 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 $B_{\text{max}}$ 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-naive 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 $B_{\text{max}}$ at present.

While future studies may demonstrate factors as the internalization of ligand-receptor complexes, and a more sophisticated accounting of possible endogenous competition, the data presented can be best explained with receptor alterations in drug-naive 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


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 $\sqrt{2}$ (ordinate/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-naive patients.


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