Further, the transfer coefficient K_3, describing the association of free ligand with the receptor sites of interest, is not a simple first-order kinetic rate constant, but represents the product of a bimolecular rate constant and the receptor concentration (*4*).

$$K_3 = K_a R \quad (5)$$

where K_a is the association rate constant for the ligand binding reaction and R represents the free receptor concentration available for binding. Only under conditions of trace receptor occupancy will K_3 become a pseudo first-order rate constant proportional to free receptor density (*4*). Under such conditions, ligand binding will reflect free rather than total receptor densities and may be modified by synaptic levels of endogenous neurotransmitter in addition to alterations in total receptor number (*6*). The maximum drug dose (200 μg) used by Wong *et al.* may be a pharmacologically significant dose in a 70-kg individual (*7*), and the assumptions regarding K_3 thus may be invalid.

The derivation of the analytic expression used by Wong *et al.* (equation 10 in (*1*)] appears to be based on several assumptions with regard to the transfer coefficients discussed above (*8*). Specifically, use of equation 10 requires that $K_1, K_2 >> K_3 >> K_4$. In the absence of direct experimental verification of these relationships, one cannot conclude that equation 10 accurately describes the experimental results.

A further issue concerns the chemical identity of the measured radiotracer in the tissue regions of interest. Wong *et al.* invoke the presence of labeled metabolites of the injected ligand within the systemic circulation, but offer no information about the presence or absence of such products within brain tissue.

While one can simplify the requisite data for in vivo receptor binding by deleting arterial blood, tissue time-activity curves, and cerebral blood flow determinations from the calculations, it has not been demonstrated that one may safely do so. We therefore urge that a more conservative approach be applied before age-related changes in ligand uptake are interpreted as reflections of altered neurotransmitter receptor density.

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8. It is further assumed that the derivation of equation 10 was via substitution of equations 7 and 8 in equation 6 [not 5, as stated in (*1*)].

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Response: Since we described the imaging of dopamine and serotonin receptors in the living human brain (*1*), we have continued to observe that the ratio of the tracer activity in the caudate nucleus and putamen to that in the cerebellum is a linear function of time for at least 120 minutes after injection of the tracer 3-*N*-[^{11}C]methylspiperone ([^{11}C]NMSP).

This observation led us to propose the ratio index for the estimation of relative receptor density (*2*). We subsequently derived a method for the measurement of absolute receptor density (*3*). The ratio index evolved from the labeling in vivo of neuroreceptors in rodents (*4*). A three-compartment model helps explain why the caudate-cerebellar ratio as a function of time can be an index of the rate of ligand binding, k_3 (*2*).

Frey *et al.* raise the issue of capillary versus arterial plasma for the model. The definitions of the constants K_1^* and K_2^* suggested by them are essentially the same as our K_1 and k_2 (*5*): K_1 is a clearance related both to blood (or plasma) flow (F) and to the permeability surface (PS) area product in the expression:

$$K_1 = F\,(1 - \exp\{-PS/F\})$$

and k_2 is the fractional clearance of the ligand from brain, defined as:

$$k_2 = K_1/\lambda$$

where λ is the distribution volume or partition coefficient of the ligand between brain and blood. The complex relation between PS product, clearance K_1, and arterial and mean capillary concentration has been previously derived. K_1 and k_2 operate on the arterial concentration at all times, from influx to steady state; hence, the reason for our choice of such constants (*5*).

We agree that $k_3 = k_{on} B'_{max}$, where B'_{max} is the quantity of receptors available for binding and k_{on} is the association rate constant (referred to as R and K_a, respectively, by Frey *et al.*) (*3, 7*). The bimolecular association constant k_{on} may change with age, but we hypothesize that a decrease in the number of available receptors is more likely. In this regard, animal and human autopsy studies have not revealed changes in affinity with age, but only changes in receptor number (*6*). Hence we attribute changes of k_3 to changes of B'_{max}. An observed change of B'_{max} can reflect a reduction of the total receptor number or a change of occupancy by endogenous or exogenous ligands, but an effect of endogenous ligands is not likely with such high-affinity ligands as NMSP.

The amount of injected [^{11}C]NMSP did not affect the rate of binding of the radioligand to the caudate or putamen with doses even greater than the tracer range of [^{11}C]NMSP. There is no evidence from [^{11}C]NMSP studies thus far that pharmacologic doses of the radioligand were used. Even in experiments with prior administration of pharmacologic blocking doses of unlabeled haloperidol, the binding of [^{11}C]NMSP was irreversible during the period of observation. Thus, there was no departure from a pseudo–first-order reaction. No significant dissociation from the receptor has ever been observed by us.

We have also employed a complete three-compartmental model that allows separate estimation of the values of K_1, k_2, and k_3 of [^{11}C]NMSP binding, as well as B_{max} (the absolute density of receptors) for [^{11}C]NMSP and the K_I (inhibitory constant) of haloperidol, a dopamine receptor blocking agent (*3, 7*). We have found that k_3 is not negligible compared to k_2. Using arterial or arterialized plasma concentrations of [^{11}C]NMSP corrected for metabolites, and two PET scans per subject (one of which was preceded by blocking doses of haloperidol), we found that human caudate B_{max} averaged 14 pmol/g, and K_I for haloperidol was 2.0 nM in young normal volunteers. These values are similar to those obtained in autopsy studies of human caudate nuclei with [^{3}H]NMSP and [^{3}H]spiperone (*3, 7, 8*).

Frey *et al.* also raise the issue of tissue

metabolites. Our estimates of B_{max} included model correction for brain as well as plasma metabolites, with calculated values found to be similar to measurements by high-performance liquid chromatography (*3, 7*). When ^{18}F-labeled NMSP was used in rodents (*9*), concentrations of metabolites in the caudate nucleus and cerebellum were quite small. In the same study the unchanging striatal radioligand mass over time suggested similar inferences about low concentrations of [^{11}C]NMSP metabolites in brain.

Administering double or multiple injections of low and much higher (blocking) doses of neuroleptic drugs makes it possible to measure absolute receptor density (B_{max}). The slope of the line relating the caudate-cerebellar ratio to time is useful as a preliminary index for the generation of hypotheses, especially in low receptor density states. To collaborate this, we note that according to our absolute density measurements, aged normal subjects over 58 years of age have B_{max} values which are approximately half that of young normal subjects (*7*). This is compatible with our results with Ca/Cb slope (*2*).

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Effects of Growth Hormone–Releasing Factor in the Brain

THE ABILITY OF GROWTH HORmone–releasing factor (GRF) directly to stimulate release of pituitary growth hormone (GH) is now well established (*1*). Intravenous (*2*), subcutaneous, and intranasal (*3*) routes of administration are known to be effective. The report by Tannenbaum (*4*) on the effects of intracerebroventricular administration of GRF on plasma GH and glucose concentrations and behavior is presented as evidence that this neuropeptide might have direct effects within the central nervous system, these being the inhibition of pituitary GH secretion, presumably by a GRF negative feedback effect on GRF itself, and the stimulation of behavioral activity. Our own observations of the effects of intracerebroventricular administration of GRF in rats are not consistent with those of Tannenbaum. Indeed, they support the argument that GRF, at the doses used, stimulates GH secretion and inhibits behavioral activity.

We have reached these conclusions using experimental models that are identical or similar to those used by Tannenbaum (*4*). They can be summarized as follows: approximately 2 weeks before experimentation, rats were surgically prepared with stainless steel guide cannulas aimed at the lateral cerebral ventrical. In addition, some animals were prepared with stainless steel recording electrodes in the calvarium over the sensorymotor and occipital cortices (*5*). Other animals were prepared with intravenous catheters in the right external jugular vein (*6*). The rats were then adapted to individual isolation recording chambers. Intracerebroventricular injections of 10 μg of human GRF (1–44)NH_2 (*7*) in saline or of saline alone were given over 30 seconds with a Hamilton syringe. To monitor the effects of GRF on plasma GH concentrations, an initial blood sample was drawn at 1000 hours, followed by the intracerebroventricular injection. Sequential blood samples were then drawn at 30-minute intervals for the next 6 hours. To monitor the effects on levels of arousal, electroencephalogram (EEG) and behavioral recordings were made before, during, and up to 120 minutes after the injections of saline or GRF. EEG analysis was made by (i) calculating the Fourier transform of 4-second continuous epochs from 0 to 127.75 Hz in Y4 Hz increments, (ii) determining the power spectra of the epochs, (iii) further compressing the data to six frequency bands, and (iv) calculating the mean power density (*8*). Behavioral analysis was made by measuring locomotor activity during the normally active (nocturnal) period. Recordings were made in cages with two infrared photocell beams. Activity was quantified by totaling photocell beam interruptions for 5-minute epochs for 4 hours after the intracerebroventricular injection.

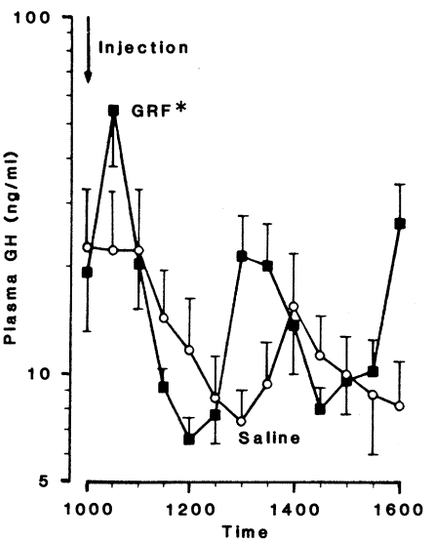

Fig. 1. Effect of intracerebroventricular administration of GRF (10 μg) or saline on GH secretion in conscious, freely moving male rats. The neuropeptide caused a significant increase ($P < 0.01$) in plasma GH concentration 30 minutes after administration. Each data point represents the mean for eight animals. Vertical lines represent the standard error of the means. GH concentrations are expressed in units of the NIH reference preparation GH-RP-2. As we used it, the preparation was ten times more potent than the reference preparation used by Tannenbaum (*4*). When corrections for this factor are made, the GH concentrations in the two studies are seen to be virtually identical.