mals with SAIDS of inversion of helper to suppressor T cell ratios determined with T_4 and T_8 monoclonal antibodies (Table 2). In this regard, the monkeys with SAIDS differed from humans with AIDS.

Numerous attempts were made to isolate viruses from the whole blood or filtered plasma that transmitted SAIDS. A variety of cell cultures were used including the continuous monkey kidney cell lines Vero and MA-104; low-passage Flow 7000 and W138 human fibroblasts, low-passage rhesus monkey kidney and lung fibroblast cells; and low-passage lung fibroblasts from the monkey *Erythrocebus patas*. No viral isolations were made from the blood or filtered plasma that caused SAIDS or from similar samples from recipient monkeys that developed SAIDS.

We previously reported that SAIDS could be experimentally transmitted to rhesus monkeys by inoculation with supernatant fluids from 10 percent homogenates (clarified by centrifugation at low speed) of organs from donor animals with SAIDS (*3*). Rhesus monkey CMV was isolated from the SAIDS-l inoculum used in those studies and from the urine of all four inoculated animals. However, SAIDS did not develop in rhesus monkeys intravenously inoculated with a high-passage laboratory strain of rhesus monkey CMV (283T) or with a recent isolate from a normal healthy animal, passed only three times in vitro (*3*). To determine whether a unique strain of CMV might be the cause of SAIDS, we inoculated two rhesus monkeys with an isolate of rhesus monkey CMV made from the SAIDS-l inoculum and passed four times in vitro in Flow 7000 human fibroblasts (*3*). These animals also did not develop SAIDS. In addition, rhesus monkeys with preexisting high titers of antibody to CMV have become infected naturally and experimentally with SAIDS. Rhesus monkey CMV was not isolated from the filtered plasma or whole blood used to transmit SAIDS, nor has it been isolated from CMV antibody negative animals receiving these inocula. These data suggest that rhesus monkey CMV is not the etiologic agent of SAIDS.

Since human T-cell leukemia virus (HTLV) has been associated with human AIDS (*5*), evidence was sought for the presence of a similar agent in SAIDS. Antibody to the p24 polypeptide of HTLV was not found by radioimmunoassay in infectious plasma from monkeys with SAIDS or in healthy rhesus monkeys housed with diseased monkeys and thus at risk of acquiring the immunodeficiency syndrome (*6*). Also, a significant reverse transcriptase activity was not detected in infectious plasma (*6*). However, these results only rule out a marked retroviremia and further studies with in vitro culture techniques are required (*6*). Type C retrovirus particles were not seen by electron microscopy in thin sections of lymph nodes or bone marrow or in cultured T cells of animals with SAIDS (*7*).

Although simian adenoviruses were not isolated from the filtered plasma or whole blood used to produce SAIDS, they have been isolated from many animals with SAIDS. Adenoviruses were isolated from all four experimentally inoculated monkeys (B-784, B-649, B-883, and B-884) from our previous study (*3*). The isolate from B-784 was typed as adenovirus type 11 (*8*). Filtered plasma from B-784 was the inoculum for monkeys 5 and 6 described in this report. An adenovirus was also isolated from the urine, feces, and kidney of monkeys 5 and 6 and also from the mesenteric node of monkey 6. Typing of these isolates has not been completed. Simian adenovirus type 23 was isolated from the feces of two rhesus monkeys that received urine from monkey B-784 (*7*). Adenovirus type 11 was also isolated from a healthy uninoculated normal control animal randomly selected from the CPRC colony, suggesting that these adenovirus isolates are opportunistic agents not etiologically linked to SAIDS.

The present studies demonstrate the experimental transmission of SAIDS with whole blood or filtered plasma. All recipients (eight of eight rhesus monkeys) developed signs of SAIDS within 2 to 4 weeks after inoculation, and (six of the eight recipients became moribund and died between 5 and 11 weeks after inoculation. We have also succeeded in transmitting SAIDS to two rhesus monkeys inoculated with pooled serum from diseased animals (data not shown). The transmission of SAIDS with infectious plasma that was passed through a 0.45-μm pore size filter provides evidence that the causative agent is small and probably a virus. These results are consistent with a recent report on transmission of a similar disease to macaque monkeys with cell-free material. However, in that study, a filtrate of lymphoma tissue was used as the inoculum (*2*). Efforts must now be focused on identifying and characterizing the etiologic agent of SAIDS. Such studies will contribute to the understanding and control of both SAIDS and human AIDS.

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Analyzing Nonlinear Scatchard Plots

The report by Paul *et. al.* (*1*) is a significant contribution to the field of receptor pharmacology. The authors described saturable and specific binding of (+)-[^{3}H]amphetamine in rat brain. However, the resolution of their Scatchard plot (figure 1A) into two apparent binding sites was done incorrectly, and consequently the kinetic constants determined are inaccurate.

The advent of radioligand receptor binding techniques and the rapid increase in the application and sophistication of receptor studies has led to a propensity for inappropriate interpretation of Scatchard-type data. Norby *et.*

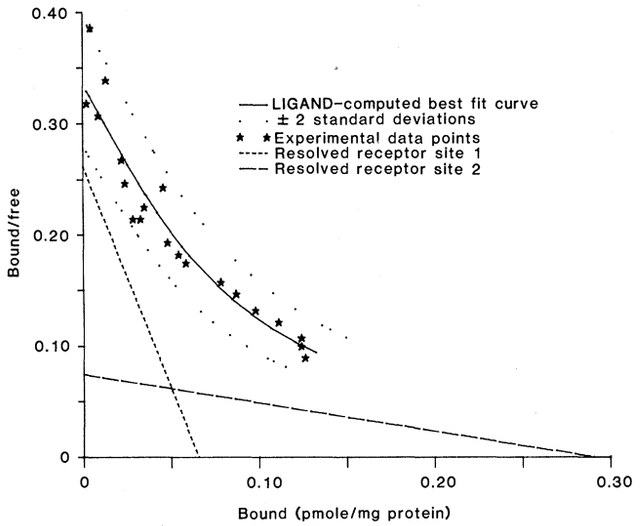

Fig. 1. Scatchard plot of (+)-[^{3}H]amphetamine binding data from figure 1A of Paul *et al.* (*1*).

al. (*2*), in a survey of papers published in selected journals since 1975, identified more than 50 papers in which nonlinear Scatchard plots were incorrectly analyzed.

A nonlinear Scatchard plot can represent two classes (independent or dependent) of binding sites; however, accurate resolution of kinetic constants for the two sites cannot be taken as simple linear regression and extrapolation of each half of the curve. It must be recognized that both receptor sites are in equilibrium and compete for the same pool of radioligand. The binding of radioligand molecules to one site effectively decreases the pool of radioligand available to the second site. It follows, then, that the site with the highest affinity for radioligand will show the highest percentage of binding at lower radioligand concentrations and, depending on the affinity differences, will approach saturation before binding to the second site becomes significant. In spite of this, the free radioligand concentration is generally measured as the total concentration at time zero minus the concentration bound at equilibrium. Graphical analysis, therefore, must separate mathematically the competition between the two binding sites for radioligand. In the report by Paul *et. al.* it is apparent that such considerations were not made when the data were analyzed.

Because of these factors, methods have been developed for the mathematical resolution of curved Scatchard plots (*3–8*). I routinely use the "LIGAND" program developed by Munson and Rodbard (*6*) to analyze receptor binding data. I extracted values for the data points presented by Paul *et. al.* in their figure 1A and applied them to the LIGAND program (Fig. 1). The points fit nicely to a model involving one labeled ligand and two classes of binding sites. Resolving out the kinetic constants of the two binding sites results in a K_d (dissociation constant) of 83.7 n*M* and a B_{max} (maximum binding) of 65.5 fmole per milligram of protein for the high-affinity site and a K_d of 1.31 μ*M* and a B_{max} of 65.5 fmole/mg for the low-affinity site. These values are obviously different from those presented by Paul *et. al.* for the high- and low-affinity sites.

Furthermore, the LIGAND-resolved data demonstrates the need for more data points at higher concentrations of (+)-[^{3}H]amphetamine to accurately characterize the low-affinity site. In general, for two-site analysis, free ligand concentrations should range from less than the K_d of the high-affinity site to greater than the K_d of the low-affinity site. The range of concentrations used by Paul *et. al.* (5 to 500 n*M*) fulfilled this requirement according to their estimate of the K_d of the low-affinity site; however, since LIGAND resolution demonstrates a K_d for this site of 1.31 μ*M*, free concentrations of radioligand greater than 1.31 μ*M* are required. Finally, as a result of the misinterpretation of the kinetic parameters for the high- and low-affinity sites, the absolute values for the K_i's (inhibition constants) presented by Paul *et. al.* in table 1 will change. They used a value of 300 n*M* for the K_d of the low-affinity site, when actually a K_d of 1.3 μ*M* is more accurate. As a result, the K_i presented in table 1 for (+)-amphetamine becomes more believable and *p*-chloroamphetamine, aminoxaphen, and fenfluramine are slightly more potent than compared to (+)-amphetamine.

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We agree with Light that the analysis of nonlinear Scatchard plots is best carried out by using computerized mathematical methods such as LIGAND (*1*). In reanalyzing our data (*2*) with this program, Light calculated an apparent K_d of approximately 84 n*M* for the "high-affinity" site and 1.3 μ*M* for the "low-affinity" site (compared to our values of 93 n*M* and 0.3 μ*M*, respectively, calculated by visual estimation of the sites followed by linear regression analysis). Light's value for the low-affinity site corresponds very well with the K_i value for displacement of (+)-[^{3}H]amphetamine binding by (+)-amphetamine presented in our table 1 (1.6 μ*M*) and resolves the fivefold discrepancy between the apparent K_d and K_i values. It should be noted, however, that the apparent K_d and B_{max} values could have as easily been calculated from displacement data such as those in our report and yield values close to those calculated by direct binding techniques.

We recently characterized the specific binding of (+)-[^{3}H]amphetamine in various subcellular fractions of rat brain. As we previously reported (*2*), specific (+)-[^{3}H]amphetamine binding is highly enriched in the synaptosomal fraction. Figure 1, a and b, show a typical saturation isotherm and Scatchard plot of specific (+)-[^{3}H]amphetamine binding in crude synaptosomal membranes from rat hypothalamus over a concentration range of 0.1 to 8.0 μ*M*. In this preparation, Scatchard analysis of specific binding yields a linear plot with only one binding site fitted with the nonlinear least-squares curve-fitting program (*1*). The apparent K_d value calculated with this program (Fig. 1b) is similar to that obtained by the method of Klotz (Fig. 1c) (*3*) ($K_d \sim 1.3$ μ*M*), and is virtually identical to the apparent K_d value for the low-affinity site calculated by Light. Furthermore, the K_i values for displacement of (+)-[^{3}H]amphetamine binding by (+)- and (−)-amphetamine (Fig. 1d) [calculated by using the common derivation of the Cheng-Prusoff equation (*4*), with an ap-

parent K_d of 1.3 μM for (+)-[^{3}H]amphetamine binding] yield values of 1.9 and 5.59 μM, respectively (N = 6), in good agreement with our original values (*2*).

Curvilinear Scatchard plots may result from a number of factors, including negative cooperativity and the presence of multiple binding sites (*5*). Although our recent data suggest only a single class of noninteracting binding sites for (+)-[^{3}H]amphetamine in synaptosomal membranes (Fig. 1), we cannot entirely rule out the presence of multiple binding sites and (or) the presence of negative cooperativity in the crude membrane preparations originally employed. Nevertheless, the demonstration of saturable and stereospecific binding sites for (+)-[^{3}H]amphetamine that (i) are unevenly distributed in various brain regions (highest density in brainstem and hypothalamus), (ii) are present on synaptosomal membranes, (iii) have an apparent K_d well within the range of pharmacologically relevant brain concentrations of amphetamine, and (iv) are correlated with the anoretic potencies of various phenylethylamines in vivo is not changed by Light's reanalysis of the data. The low to undetectable levels of specific (+)-[^{3}H]amphetamine binding in peripheral tissues such as liver, kidney, and heart further suggest that these sites are related to amphetamine's neuropharmacological actions (*6*). Moreover, both the apparent K_d and B_{max} values for specific (+)-[^{3}H]amphetamine binding (as with even the best-characterized binding sites) are only estimates. In the case of (+)-[^{3}H]amphetamine binding, several variables, including the preincubation and incubation temperature, ionic conditions, and tissue preparation, greatly influence the apparent K_d and B_{max} values (*6*). Such factors will change the apparent B_{max} by at least one order of magnitude. Thus a three- to fourfold difference in the apparent K_d value, as calculated by nonlinear least-squares curve-fitting analysis (as opposed to linear regression analysis), does not significantly alter the conclusions of our report.

The analysis of binding data by any mathematical method rarely substantiates or refutes the pharmacological or biological significance of a binding site. The ultimate significance of our findings will be determined by more detailed structure-activity studies of other compounds.

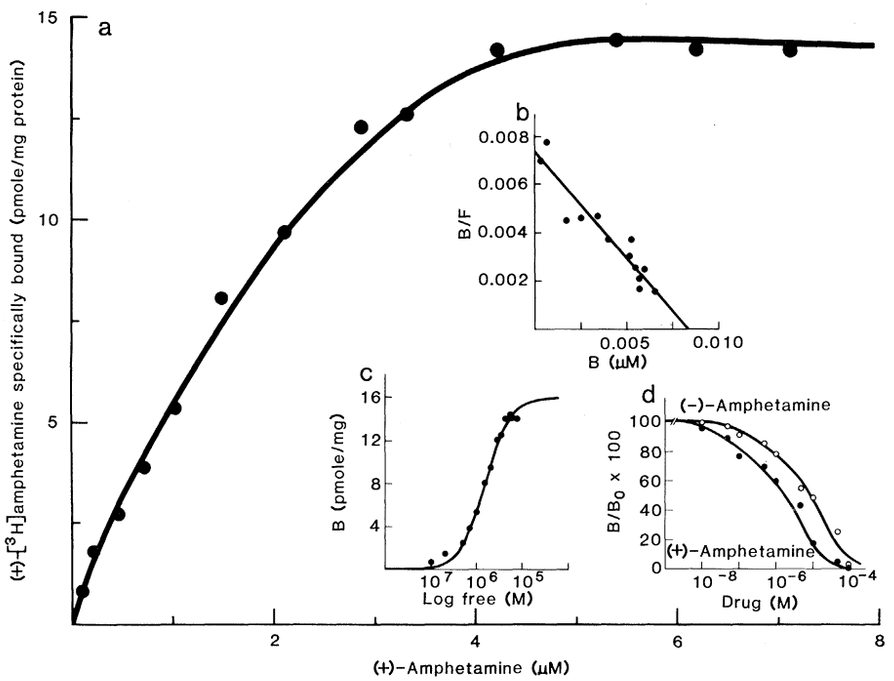

Fig. 1. Specific binding of (+)-[^{3}H]amphetamine to crude synaptosomal membranes (P_2 fraction) from rat hypothalamus. Membranes were prepared from hypothalami of male Osborne-Mendel rats (120 to 150 g). Brain tissue was pooled and disrupted in 40 volumes (weight to volume) of ice-cold 0.32M sucrose by a Teflon-glass homogenizer. The homogenate was centrifuged at 1000g for 10 minutes and the supernatant was centrifuged at 20,000g for 30 minutes. The resulting pellet was gently resuspended in ice-cold 50 mM tris-HCl (pH 7.4) and centrifuged at 20,000g for 10 minutes. The final pellet was resuspended in 40 volumes (25 mg/ml) of ice-cold 50 mM tris-HCl (pH 7.4) with a Brinkmann Polytron. Specific (+)-[^{3}H]amphetamine binding was measured exactly as in (*2*). (a) Saturation isotherm of specific binding over a ligand concentration of 0.1 to 8.0 μM. (b) Scatchard plot of the data in (a), obtained by the nonlinear least-squares curve-fitting program of Munson and Rodbard (*1*), yielding an apparent K_d of 1.34 ± 0.21 μM and a B_{max} of 11.0 ± 1.2 pmole/mg. (c) The same data analyzed according to Klotz (*3*). The concentration of bound ligand is plotted against the log of free ligand. The apparent K_d and B_{max} values, calculated with the computer curve-fitting program ALL FIT (*7*), are 1.49 μM and 16.0 pmole/mg, respectively. (d) Representative displacement curve of specific (+)-[^{3}H]amphetamine binding by (+)- and (−)-amphetamine. K_i values were calculated by the formula $K_i = IC_{50}/1 + ([\text{ligand}]/K_d)$, where IC_{50} represents the concentration of drug necessary to inhibit 50 percent of specific binding, [ligand] was 0.1 to 0.2 μM, and the apparent K_d was 1.3 μM. K_i values in six separate experiments were 1.9 ± 0.6 and 5.59 ± 1.6 μM (means ± standard errors) for (+)- and (−)-amphetamine, respectively ($P < 0.05$, paired t-test); B, bound; F, free; $B/B_o \times 100$, percent specific binding in the presence of drug compared to no drug.

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