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Letter to the Editor

Concentration of Dopamine Transporters: To B_{max} or Not to B_{max}?

We read with interest the paper by Logan et al. (1997) entitled "Concentration and Occupancy of Dopamine Transporters in Cocaine Abusers With [11C]Cocaine and PET" in the December issue of *Synapse*. The conclusion of the Logan paper was that estimates of the available number of receptor sites, B'_{max}, from PET data are inherently unreliable and should, in general, be avoided in favor of the more robust measure, distribution volume ratio ($DVR = B'_{max}/K_D + 1$). They based their conclusions primarily on the variability that they found in B'_{max} using either the pseudo-equilibrium (PSE) method (Farde et al., 1989) or nonlinear least-squares (NLSQ) fitting of the three-compartment model as compared to the variability in DVR calculated from their own graphical method (Logan et al., 1990). The Farde method has been suspect for some time and, as Logan et al. show, it is highly sensitive to the choice of particular pseudo-equilibrium point. Neither are we surprised about the poor performance of the NLSQ method for estimating B'_{max} from a single injection, for reasons stated below, and we applaud Logan et al. for demonstrating the shortcomings in these methods. However, as support for their position against using B'_{max}, they also cited variability that we observed recently (Morris et al., 1996a) in density of the dopamine transporter (DAT) in rhesus monkeys. In that study we used a more sophisticated and theoretically sound technique of three injections of ^{11}C-CFT followed by NLSQ fitting of all of the dynamic data simultaneously. Logan et al. further reasoned that B'_{max} should correlate with B'_{max}/K_D. Since in the results for normal animals in our paper it did not, they asserted that the only parameter that we have really measured reliably is B'_{max}/K_D, which was fairly well preserved across 3 experimental animals. Unfortunately, the Brookhaven group ignored more compelling intra-subject data (see animal A) in our paper, which showed that when pre-synaptic neurons were destroyed by MPTP, both the animal's B'_{max} (122 nM pre-MPTP, 13 nM post-MPTP) and B'_{max}/K_D (3.5 pre-, 0.28 post-) dropped precipitously, whereas the K_D remained nearly constant (34 nM pre-, 46.6 nM post-). Thus, when a (MPTP) lesion was specifically directed at the density of transporters on neurons terminating in the striatum, the change in parameter values (in repeated measures) reflected this fact.

We fear that a consequence of the recent Logan paper will be to improperly cast aspersions on a valid and important technique for estimating B'_{max}, k_{on} and k_{off}, separately ($K_D = k_{off}/k_{on}$), from dynamic PET data. As such, we wish to respond on methodological and mechanistic grounds. First, we maintain that for certain ligands, it is possible to estimate B'_{max} and K_D separately using a multiple-injection strategy, and there are instances when this is highly advisable. Examination of a patient group under treatment with agonist drugs that cause receptors to change from high to low affinity state would be one such instance. Second, the fact that B'_{max}/K_D is fairly constant across healthy subjects does not necessarily indicate that it alone is the parameter of physiological significance nor should inter-subject variability of B'_{max} and its possible causes, which we discuss below, be taken as proof that it is wrong.

THEORY OF MULTIPLE-INJECTION (M-I) TECHNIQUE

Classic methods of in vitro receptor assay are based on measurements at several specific activities (SA), with two measurements being the minimum required to characterize a simple receptor system. The use of three sequential injections of PET radioligand and the analysis of all data with a compartmental model, which accounts for the radioactive and non-radioactive species independently, was first proposed by Delforge et al. (1989). The intent was to estimate all the model parameters, especially the three related to binding, B'_{max}, k_{on}, and k_{off}, from a single experiment. Qualitatively, the three injections of radioligand at alternating high and low SA can be considered analogous to the in vitro techniques that are the gold standard for receptor assay. Multiple injections are used to vary SA, thereby increasing the information content of the PET curves with respect to the receptor density, affinity, and nonspecific binding. With appropriately designed experiments, one can identify the unique

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parameter set that produces a predicted PET curve to describe the system at multiple set-points (i.e., to fit all the data). From a theoretical standpoint, the question is one of parameter identifiability (see for example, Jacquez, 1985 or Carson et al., 1983). In other words, is the structure of the chosen model such that the parameters can be identified unambiguously from experiments?

Why Does It Work?

Following Delforge et al. (1989, 1990) and others (Mintun et al., 1984; Huang et al., 1986), the PET activity can be modeled as the weighted sum of the activities in the plasma, free and bound "compartments" of a brain region. To know

$$\boldsymbol{S} = \begin{bmatrix} \frac{\partial PET_{t_0}}{\partial K_1} & \frac{\partial PET_{t_1}}{\partial K_1} & \cdots & \frac{\partial PET_{t_n}}{\partial K_1} \\ \vdots & \vdots & \ddots & \cdots \\ \frac{\partial PET_{t_0}}{\partial k_{on}} & \frac{\partial PET_{t_1}}{\partial k_{on}} & \cdots & \frac{\partial PET_{t_n}}{\partial k_{on}} \\ \frac{\partial PET_{t_0}}{\partial k_{off}} & \frac{\partial PET_{t_1}}{\partial k_{off}} & \cdots & \frac{\partial PET_{t_n}}{\partial k_{off}} \\ \frac{\partial PET_{t_0}}{\partial B'_{max}} & \frac{\partial PET_{t_1}}{\partial B'_{max}} & \cdots & \frac{\partial PET_{t_n}}{\partial B'_{max}} \end{bmatrix} \quad (1)$$

whether the parameters are identifiable, we must consult the sensitivity matrix. The sensitivity matrix is the matrix of derivatives of the PET activity with respect to the various model parameters evaluated at all of the time-points at which data are acquired during the experiment. The rows of this matrix are the sensitivity coefficients. They are the time-varying curves that indicate how the PET activity would change over time with incremental changes in each of the parameters. If this matrix is not of full rank, that is, if any two or more of the sensitivity coefficients are linearly dependent, then these parameters cannot be determined from the given model and experiment. As an example, we display (Fig. 1) the respective sensitivity coefficients for k_{on}, k_{off}, and B'_{max}, based on a model-fit of the ^{11}C-CFT data in our *Synapse* paper. The three curves (which represent the last three rows of the matrix in equation 1) are admittedly very similar following the first and second injections. This suggests that neither a single bolus (as tested by Logan with the NLSQ method), nor a two-injection (high and low SA) protocol would be sufficient to identify the three binding parameters. However, they are distinguishable thanks to data from the third injection. Specifically, after the third injection at 90 minutes, the PET activity could be expected to (1) not be affected by larger k_{on}, (2) be diminished by larger (negative) excursions in k_{off} values, and (3) increase with larger B'_{max} values. Thus, the distinct contributions of the three parameters can be determined if the total response to three injections of radioligand is examined.

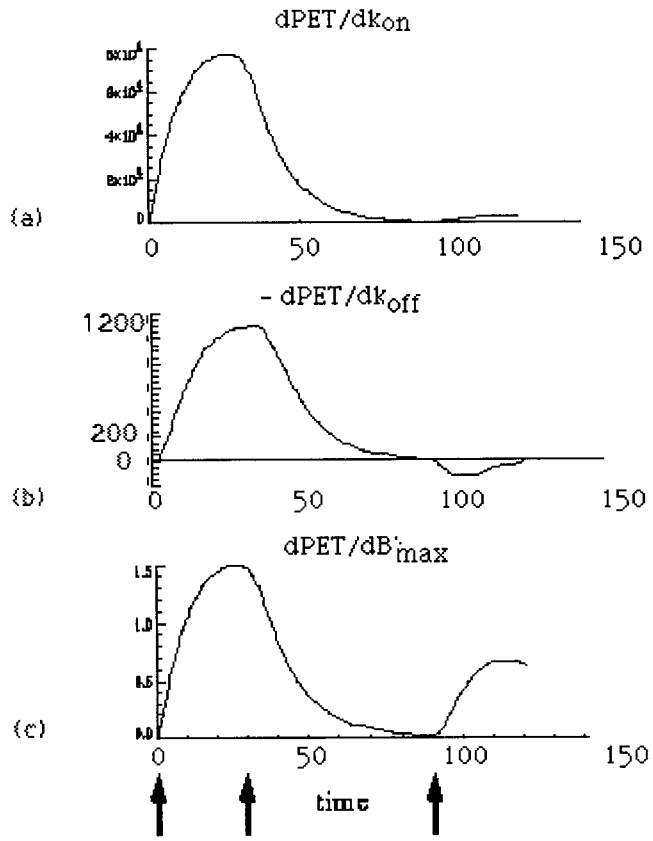

Fig. 1. **a–c:** Sensitivity coefficients with respect to k_{on}, k_{off}, B'_{max}, respectively, vs. time (in minutes). Vertical arrows at bottom correspond to the timing of the three injections of labeled CFT (the derivative with respect to k_{off} has been inverted.). Notice the overriding similarity between each of the three derivatives for the first 90 minutes and the different behavior of the three curves *following* the third injection. This comparison of sensitivity curves lends support to the idea of separating the effects on the PET signal (i.e., identifying) k_{on}, k_{off}, B'_{max} from a three-injection protocol.

LIMITATIONS OF MULTIPLE-INJECTION TECHNIQUE IN PRACTICE

Identifiability is a practical issue as well as a theoretical one. The curves shown in Figure 1 do not consider error in the data measurements. In our experience, there exists a particularly tricky experimental source of uncertainty in B'_{max} estimates from M-I studies. Recall, that we employed the Delforge model to analyze our M-I data. We have previously discussed at great length the strength of the Delforge model for M-I data (Morris et al., 1996b). Unlike models that merely track the behavior of the labeled species, the Delforge model correctly predicts the percentage of available receptor

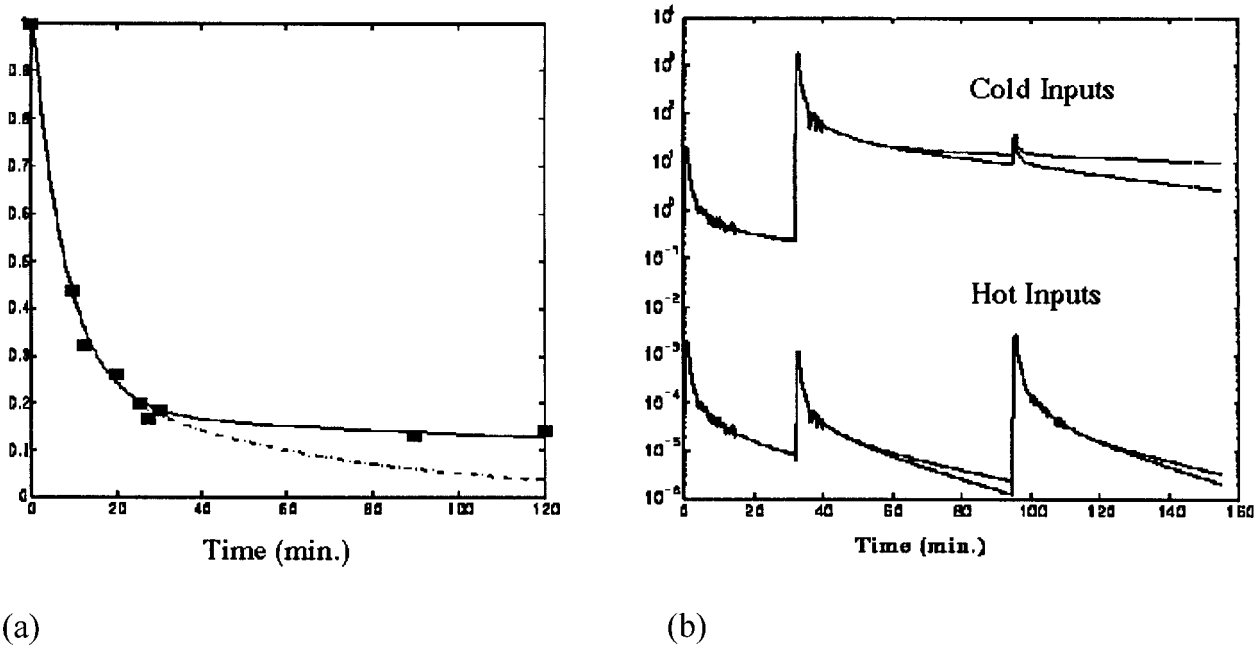

Fig. 2. **a:** Fraction of blood radioactivity as unmetabolized ligand following a single bolus injection of ^{11}C-CFT. Single (solid line) and double exponential (dotted line) fits to 30 and 120 minutes of metabolite data, respectively. **b:** Pairs of Hot and Cold input functions (log scale, pmol/ml) for a multiple-injection study reconstructed after correction for metabolites via each of the curves in a. Correction via the single-exponential metabolite curve results in slightly lower Hot and Cold curves, which diverge from the properly corrected curves at late time.

sites at any time during the study. It does so by accounting for the labeled and unlabeled species explicitly in all compartments rather than by inferring the action of the unlabeled species at the receptor from knowledge of the radioactive species everywhere and the ratio of labeled to unlabeled species in the plasma compartment. To maintain what are essentially two parallel models of the labeled and unlabeled species, coupled by their saturable interaction at the receptor, the technique depends on two input functions: one for hot, one for cold. Unfortunately, this may also be the Achilles heel of the technique.

Why Don't Multiple Injections and the Delforge Model Work Perfectly?

Of the two input functions needed for the Delforge model, the one that specifies the concentration of cold ligand in the plasma at any time is the more problematic. Because it is not measured directly, it must be constructed from knowledge of the hot input and a model relating hot to cold. This "model" is, of course, a scaling of radioactive counts from respective injections based on their SA (see appendix A of Morris et al., 1996a). Consider, however, that as soon as more than one injection has been introduced, determining which radioactivity in the plasma came from which injection (and hence, which SA to apply) is not completely straightforward. One approach, that we employed, is to assume that each constituent part of the compound input function has the same shape. In other words, that each injection of ligand is removed from the plasma compartment identically. Armed with a canonical plasma curve, one can predict how much cold ligand is in the blood at any time. On top of this parameterized version of the hot plasma input curve, it is also necessary to apply metabolite correction. Again, a model or canonical curve describing metabolic loss of native ligand from the plasma over time is applied. Unfortunately, metabolite measurements are difficult at late times owing to rapid loss of radioactivity (particularly for 11C-labeled ligands like CFT and cocaine) and so the metabolite curve is often an extrapolation from early-time measurements. We have found that small differences in how or for how long the metabolites are measured can lead to sizable differences in the predictions for B'_{max} (Bonab et al., 1995). Figures 2 and 3 show an instance of this susceptibility of B'_{max} to differences in the duration of metabolite data acquisition. In Figure 2a fitted metabolite corrections are determined from either 30 minutes (dotted lines) or all 120 minutes of metabolite data (solid lines). These slight differences in the metabolite curve lead to seemingly negligible differences in the metabolite-corrected hot plasma curves (Fig. 2b, bottom). Nevertheless, the two different *hot* plasma curves lead to noticeable late-time differences in the reconstructed *cold* plasma curves. These differences, in turn, lead to indistinguishable fits to the PET data (see Fig. 3) but significant variability in the

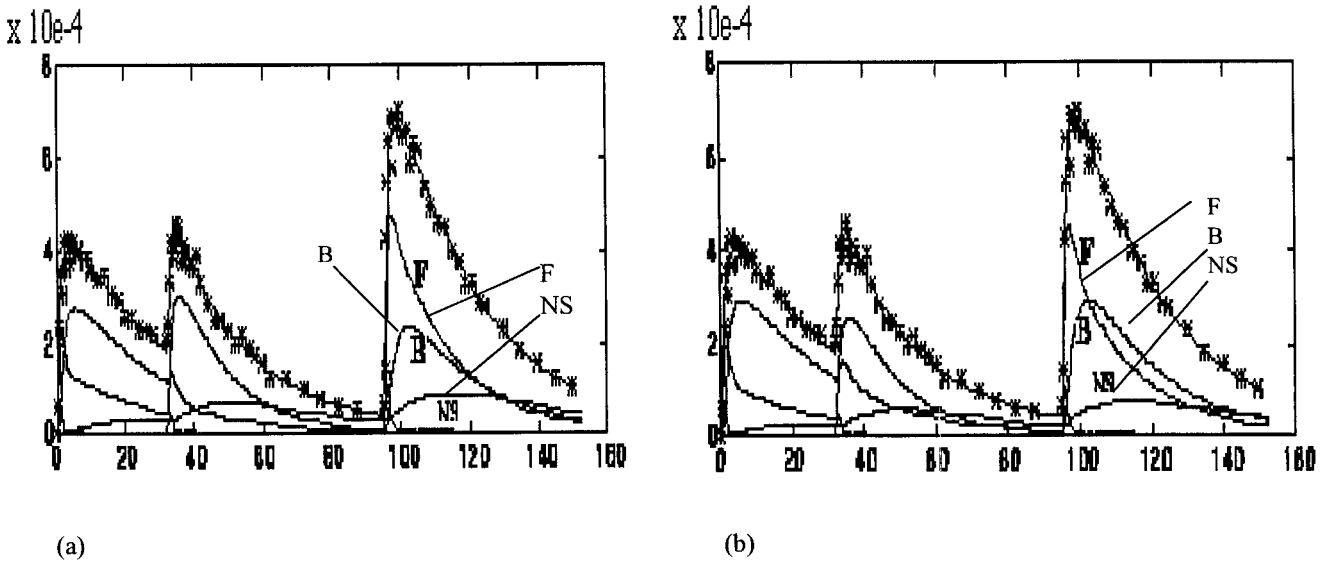

Fig. 3. **a:** Fitted test PET data (pmol/ml) using metabolite correction based on the first 30 minutes of metabolite data. **b:** Curve fitted to same multiple-injection data using 120 minutes of metabolite data. Although the fits are identical, use of the correct metabolite curve properly predicts a larger bound component (labeled B) of the tissue radioactivity. F and NS refer to free and nonspecifically bound ligand concentrations predicted by the model.

estimates of B'_{max}: 70 pmol/ml using 30 minutes of metabolite data vs. 124 pmol/ml using all the metabolite data. In this simulated data set, the true B'_{max} was 122 pmol/ml. By examining the individual compartment concentrations (F, B, NS) in Figures 3a and b, we see that the exact shape of the metabolite curve—and hence the plasma curves—exerts its bias on the B'_{max} estimate by forcing a trade-off between the predicted amounts of ligand in the free and bound states.

In retrospect, some of the inter-subject variability in B'_{max} estimates reported earlier (Morris et al., 1996a) is likely to have resulted from the questionable application of a population-average metabolite correction function across all subjects. Matching metabolite measurements on individuals with their respective plasma data could reduce that variability. (The metabolite data would probably have to be acquired on a separate day from the PET study so that late time points could be taken.) Additional innovation in constructing a cold blood curve from a hot curve (different constraints, different weighting schemes) or direct measurement of the "cold" ligand by use of a non-positron emitting label might improve the overall precision of the method. Formal use of optimal experiment design might also improve the precision of parameter estimates (Morris et al., 1995; Muzic et al., 1996).

We have tried to demonstrate briefly the pros (and cons) of the M-I method of determining binding parameters from PET because it is important to recognize the differences between the M-I technique and some other simpler, but perhaps less accurate methods of estimating B'_{max} and/or K_D. In fact, we have demonstrated previously that graphical estimates of DV increase with increasing noise (Hsu et al., 1997), that is, they are a biased estimator of distribution volume.

BIOLOGICAL CONSIDERATIONS

We sought to establish the feasibility of quantifying a depletion in dopaminergic terminals by means of monitoring the density of pre-synaptic DAT sites in monkey striata. In that study, a dramatic depletion in dopaminergic projections (via MPTP), which was sufficient to effect gross motor deficits, was well reflected by an apparent drop of 90% in B'_{max}, whereas K_D was found to be stable (apparent increase of 20%). Because there is some evidence that K_D can change with age (Suhara et al., 1991) or exposure to agonist drugs, it would be inadvisable to use B'_{max}/K_D (or DVR) in all circumstances as an indicator of B'_{max}. In those animal studies that can be informed by autopsy studies, it is also of value to be able to compare B'_{max} and K_D values obtained in vivo and in vitro.

Homeostasis or Correlation?

As to the apparent constancy of BP (DVR-1), that is, the apparent inverse correlation between B'_{max} and K_D in our normal animals, could this not be a portrayal of biological homeostasis? We suggest that B'_{max} may be quite variable across animals but the range of binding potentials that are compatible with normal, healthy dopaminergic function is more narrow. This might lead one to investigate only the BP value. But what of the pathological case? What is the mechanism behind the

pathology? Is it a loss of receptors because of a problem of synthesizing them? Is it a loss of receptors because of a loss of nerve terminals. Is it a degeneration of the extracellular environment that causes a change in affinity between dopamine and its receptors? Because the answer to these questions may have some implication for treatment, it would seem imprudent to sacrifice any possibility of answering them by always settling for B'_{max}/K_D when B'_{max} and K_D may be at hand.

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