Brief Communication

Measuring drug occupancy in the absence of a reference region: the Lassen plot re-visited

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Quantitative estimation of neuroreceptor occupancy by exogenous drugs using positron emission tomography is based on the reduction in the total volume of distribution (Vₜ) of site-specific radioligands after drug administration. An estimate of the distribution volume of free and non-specifically bound radioligand (V₉ND) is also required to distinguish specific from total binding. However, a true reference region, devoid of specific binding, is often not available. We present a transformation of a graphical method, originally introduced by Lassen, using regional estimates of Vₜ alone to determine occupancy, together with an extension that does not require baseline data. Journal of Cerebral Blood Flow & Metabolism (2010) 30, 46–50; doi:10.1038/jcbfm.2009.190; published online 9 September 2009

Keywords: graphical analysis; occupancy; PET

Introduction

The estimation of neuroreceptor occupancy by an exogenous drug using positron emission tomography (PET) is based on the measurement of the fractional decrease in the specific binding potential (BP) of a radioligand after drug administration. The BP can be derived from the total volume of distribution of the radioligand (Vₜ) if an estimate of the volume of distribution of the free plus nonspecifically bound radioligand (V₉ND) is available (Mintun et al, 1984; Innis et al, 2007). It is, however, frequently the case that a suitable reference region, with negligible specific binding, is not available. In 1995, Lassen et al introduced a plot based on regional changes in Vₜ after drug administration, from which occupancy could be derived despite the absence of a true reference region. The assumptions were minimal, simply that there is a range of regions with differing target density (Bₘ₉), nonspecific binding is homogeneous and occupancy is the same in all regions. This approach was originally applied to estimate regional Bₘ₉ and Kᵞ for the benzodiazepine receptor ligand, Flumazenil, over a range of drug concentrations, using radiolabeled [¹⁴C]Flumazenil under steady state conditions. The principle is, however, equally applicable to heterologous competition between tracer and drug with Vₜs derived either from quantitative equilibrium studies or from kinetic compartmental analyses of dynamic studies, under the assumption that there is a steady state of occupancy for the duration of the scan.

We find it surprising that, to our knowledge, little use of Lassen’s plot has been made (Gjedde and Wong, 2000; Asselin et al, 2003; Pinborg et al, 2007) even with the dramatic increase in the number of occupancy studies being performed with exogenous drugs and the increasing number of targets for which ligands with suitable reference regions do not exist. This contrasts with the rapid uptake of both the Patlak Plot (Patlak et al, 1983) and Logan Plot (Logan et al, 1990) for the analysis of irreversible and reversible PET data and their associated reference tissue derivatives (Patlak and Blasberg, 1985; Logan et al, 1996).

We present a simple variation on the plot presented by Lassen that we have found particularly useful in the visualization and interpretation of drug occupancy studies, together with a generalization of the method applicable under conditions where a true baseline scan in the absence of the drug is not available. In general, graphical analyses may lead to noise-induced bias in the parameter estimates if there is correlated noise present in both the x- and y-variables (Cunningham, 1985; Carson, 1993; Slifstein and Laruelle, 2000). We have therefore investigated this phenomenon in this case.
Materials and methods

Theory

Graphical methods for the analysis of occupancy data can be derived from equations for \( V_c \). Given estimates of \( V_c \) from \( n \) regions, for both baseline and post-drug scans, then,

\[
V_{\text{Base}}^T = V_S + V_{ND}
\]

\[
V_{\text{Drug}}^T = V_S(1 - O^{\text{Drug}}) + V_{ND}
\]

where \( V_{\text{Base}}^T \) and \( V_{\text{Drug}}^T \) are vectors of the regional distribution volumes obtained at baseline and post-drug administration, respectively, \( V_S \) is a vector of the regional specific distribution volumes, \( V_{ND} \) is the nondisplaceable distribution volume, and \( O^{\text{Drug}} \) is the fractional target occupancy post-drug. The implicit assumptions in equations (1) are that \( V_{ND} \) and \( O^{\text{Drug}} \) are the same for all regions. A simple rearrangement of the equations in (1) yields,

\[
V_{\text{Base}}^T - V_{\text{Drug}}^T = O^{\text{Drug}}(V_{\text{Base}}^T - V_{ND})
\]

which is the axes-transformed ‘Lassen Plot’ (Lassen et al, 1995). When represented graphically \((x = V_{\text{Base}}^T, y = V_{\text{Drug}}^T)\) this produces a linear relationship with \( x \) intercept equal to \( V_{ND} \) and gradient equal to \( O^{\text{Drug}} \).

If we make a further assumption

\[
O^{\text{Drug}} = \frac{C}{C + IC_{50}}
\]

where \( C \) is the free drug concentration at the target site and \( IC_{50} \) is the half-maximal inhibitory concentration, it is possible to derive a more general equation that does not require the existence of a baseline scan. This is equivalent to a Hill coefficient of 1 and assumes that full occupancy of the receptor sites would be approached at high drug concentrations. Under these assumptions, the following equations for two scans performed at drug concentrations of \( C' \) and \( C'' \) can be derived,

\[
V_{\text{Base}}^{T'} = V_S\left(1 - \frac{C'}{C' + IC_{50}}\right) + V_{ND}
\]

\[
V_{\text{Base}}^{T''} = V_S\left(1 - \frac{C''}{C'' + IC_{50}}\right) + V_{ND}
\]

Rearrangement of these equations yields,

\[
V_{\text{Drug}}^{T'} = V_S\left(\frac{IC_{50}}{C' + IC_{50}} - \frac{IC_{50}}{C'' + IC_{50}}\right)
\]

\[
V_{\text{Drug}}^{T''} = V_S\left(\frac{C''}{C'' + IC_{50}} - \frac{C'}{C' + IC_{50}}\right)
\]

Substitution of \( V_S \) and further rearrangement yields,

\[
(V_{\text{Drug}}^{T'} - V_{\text{Drug}}^{T''}) = \left(1 - \frac{C' + IC_{50}}{C'' + IC_{50}}\right)(V_{\text{Drug}}^{T'} - V_{ND})
\]

\[
(V_{\text{Drug}}^{T'} - V_{\text{Drug}}^{T''}) = \left(\frac{C'' - C'}{C'' + IC_{50}}\right)(V_{\text{Drug}}^{T'} - V_{ND})
\]

leading to,

\[
(V_{\text{Drug}}^{T'} - V_{\text{Drug}}^{T''}) = \frac{C'' - C'}{C'' + IC_{50}} = O^2(V_{\text{Drug}}^{T'} - V_{ND})
\]

where \( O^2 \) is the fractional occupancy by the drug in the second scan.

When represented graphically \((x = V_{\text{Drug}}^{T'}, y = (V_{\text{Drug}}^{T'} - V_{\text{Drug}}^{T''})\) \(\frac{C'' - C'}{C'' + IC_{50}}\) produces a linear relationship with the \( x \) intercept equal to \( V_{ND} \) and the gradient equal to \( O^2 \).

Another requirement here is the knowledge of the concentration of the drug at the target site. In practice, available data may only relate to the concentration of the cold drug in plasma, or to the administered dose of the drug, which allows for parameterization in terms of either the IC\(_{50}\) or ED\(_{50}\), respectively.

Examples from In Vivo Positron Emission Tomography Data Sets

To show the application of the modified and extended Lassen plots described in equations (2) and (6) we have applied them to two PET occupancy data sets acquired in vivo with the 5HT\(_{1A}\) radioligand \[^{11}\text{C}\]WAY100635; Data Set I: an \[^{11}\text{C}\]WAY100635 study in the baboon brain at baseline and 1 h after i.v. administration of a 5HT\(_{1A}\) antagonist at doses of 1.5, 10, and 150 \(\mu\)g/kg. Briefly, for each of the four scans, dynamic blood, metabolite, and tissue data were acquired for 90 min and analyzed with a two-tissue compartment model to derive regional estimates of \( V_T \) for cingulate, frontal, temporal, parietal and occipital cortices, cerebellum, hippocampus, striatum, and dorsal raphe nuclei. Data Set II: an \[^{11}\text{C}\]WAY100635 study in the human brain at baseline and after p.o. administration of 30 mg of the 5HT\(_{1A}\) partial-agonist pindolol. Data were taken from subject 5 (PET scans 1 and 4) in Martinez et al (2001). Briefly, for each scan, dynamic blood, metabolite, and tissue data were acquired for 100 min and analyzed with a two-tissue compartment model to derive regional estimates of \( V_T \) for cerebellum, dorsolateral prefrontal cortex, medial prefrontal cortex, orbito-frontal cortex, subgenual prefrontal cortex, anterior cingulate cortex, temporal cortex, insular cortex, parietal cortex, occipital cortex, medial temporal lobe, and dorsal raphe nuclei.

The graphical analysis defined in equation (2) was applied to both Data Sets I and II. In addition, the extended graphical method in equation (6) was applied to Data Set I without using the baseline data. Although the cerebellum is accepted as a reference region for \[^{11}\text{C}\]WAY100635 imaging of the 5HT\(_{1A}\) receptor, it was omitted from the regression analyses of Data Set I, so that it could be used to validate the estimation of \( V_{ND} \) in the absence of reference region data.

Simulations to Investigate Sensitivity to Noise

A series of Monte-Carlo simulations were performed to assess any noise-induced bias associated with estimation of occupancy by equation (2). The simulation was set up to consider six separate regions with the same \( V_{ND} = 2 \) and differing receptor densities, which corresponded to BP\(_{ND}\) values of \(1, 1.5, 2, 2.5, 3, 3.5\) and resultant total distribution volumes of \(4, 5, 6, 7, 8, 9\).

A series of Monte-Carlo simulations were performed by generating total distribution volume data for baseline and
post-drug scans corresponding to occupancy levels of 0%, 20%, 40%, 60%, 80%, and 100%. For each occupancy and noise level (low and high), 1000 noisy realizations were generated by adding Gaussian distributed noise to the total distribution volume. The magnitude of the noise was proportional to $V_T$ and had a standard deviation of 5% (low noise) or 10% (high noise) of the total $V_T$, corresponding to that obtained from good and moderate radioligands, respectively. Within each simulation, every pair of baseline and post-drug data ($N=1000$) was analyzed by using standard linear regression analysis of equation (2) to derive

1000 estimates of the occupancy and $V_{ND}$ at each occupancy and noise level.

**Results**

The application of the graphical analyses defined by equations (2 and 6) to the $[^{11}C]$WAY100635 study in the baboon brain after administration of a 5HT$_{1A}$ antagonist is illustrated in Figures 1A and 1B, respectively.

The graphical analysis in Figure 1A illustrates a clear linear relationship for data at each of the different doses with estimated $V_{ND}$ values (0.53, 0.10, −0.60) compared with that of the superimposed cerebellum (0.65) and derived occupancy estimates (26%, 76%, 97%) compared with those calculated independently using the cerebellum as reference (mean values 33%, 82%, and 97%). In practice, when more than one occupancy scan is available within the same individual, it may be better to constrain all x axis intercepts to be equal as part of the fitting process.

In Figure 1B, the baseline data are omitted and occupancy estimates are derived for the two highest dose levels using equation (6). A linear relationship was observed at each of the two-dose levels with estimated $V_{ND}$ values (0.54, 0.17) compared with the superimposed cerebellum (0.65) and derived occupancy estimates (80%, 97%) in agreement with those estimated using baseline data (76%, 97%).

The application of the graphical analyses defined by equation (2) to the $[^{11}C]$WAY100635 study in the human brain after administration of the 5HT$_{1A}$ partial-agonist pindolol, and now including the cerebellum, is illustrated in Figure 1C. The point corresponding to the dorsal raphe nuclei lies off the main line of regression, consistent with the interpretation that differential occupancy is achieved at the somatodendritic (dorsal raphe nuclei, 75%) and postsynaptic (other regions, 44%) 5HT$_{1A}$ receptor.

![Figure 1](image-url)
The above examples using [11C]WAY100635 were chosen, as a ‘true’ reference region was available for comparison, and also because data were available at more than one dose. However, in this example, \( V_{ND} \) was extremely low. To show the general applicability, it is important to consider the situation where nondisplaceable binding is higher. In the simulation study reported below, a larger value of \( V_{ND} \) (= 2) was therefore used.

Monte-Carlo simulations to investigate the magnitude of any noise-induced bias in the graphical estimation of occupancy are summarized in Figure 2. It is evident that there is a tendency for a small positive bias in estimates of occupancy and that this bias is more pronounced at lower occupancy levels and in the presence of increased noise. \( V_{ND} \) was poorly identified at low occupancies (%COV > 100% at occupancies < 20%), when there is little change in signal between baseline and post-drug scans, but was well identified at higher occupancies (%COV < 10% at occupancies ≥ 40%).

**Discussion and summary**

The graphical representation of occupancy studies, based on regional estimates of \( V_T \) and changes in \( V_T \) after drug administration leads to a linear relationship across regions of interest with differing BPs under the assumption that the fractional occupancy is the same across regions. This allows an easily interpretable and quantifiable estimate of occupancy from the slope and of \( V_{ND} \) from the x intercept in either the presence or the absence of a true reference region. In the former case, the plot may help to identify the appropriateness of a reference region, as in the case of the cerebellum that lies on the x intercept in the [11C]WAY100635 study illustrated here. In the latter case, it avoids the need for a full-blocking study, which may not always be possible, to estimate \( V_{ND} \). Furthermore, deviations from the model assumptions are readily identifiable. For example, regional outliers may indicate a true difference in occupancy in the region, as in the 5HT1A occupancy in the dorsal raphe nuclei by the partial-agonist pindolol (Figure 1C). If the plot deviates from linearity at high \( V_T \) values, this may indicate that the ligand is not sufficiently reversible to provide a quantitative outcome measure in the high-binding regions.

By further incorporation of an occupancy model—in this case a simple competitive binding model, it was possible to extend the method to cover the case where baseline data are not available. This implies an additional assumption that full occupancy of the target would be approached at high drug concentrations. This assumption relates principally to the selectivity of the radiotracer, and might not be valid were the radiotracer to bind significantly to more than one receptor type to which the competing cold drug might not bind. In the example here, the specific binding of the radioligand ([11C]-WAY-100635) is related to a single-binding site (the 5HT1A receptor), which has been demonstrated by the original manuscripts describing this PET ligand. The compound we examined is a pan-5HT blocker (1A, 1B and 1D); however, the binding of the compound to 1B and 1D are irrelevant in this context, as [11C]-WAY100635 has no significant binding to either of the alternative targets.

This could be applied within a population where it may not be possible to scan in the absence of the drug, but where a limited range of drug dosing would allow estimation of drug occupancy—for example, estimation of drug occupancy when access to a drug naive population is difficult. A further application of this general model is in the case where a specific radioligand displays near irreversible PET kinetics.

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Figure 2 Monte-Carlo simulation to assess noise-induced bias of the graphical occupancy analysis; (A) definition of noise free total and nondisplaceable distribution volumes for the six regions of interest and (B) occupancy estimates derived at low (5%) and high (10%) noise levels from 1000 noisy realizations at occupancy levels of 0, 20, 40, 60, 80, and 100%. Error bars correspond to 1 s.d.
A comparison of regional $V_{rs}$ at two different drug doses may result in more accurate estimates of occupancy resulting from improved numerical identification in partially occupied scans.

We have investigated noise-induced bias in the graphical approach, with a particular configuration of regions and BPs, using standard linear regression. These results should generalize to the extended plot (equation 6) and to other region and binding configurations, although the magnitude of the bias should be assessed for particular scenarios. If bias is deemed problematic, then alternative estimation methods can be applied, taking into account noise on both axes, such as total least squares (Varga and Szabo, 2002) and likelihood estimation (Ogden, 2003).

In summary, the elegant graphical method, originally introduced by Lassen et al., provides an attractive means to analyze drug occupancy studies lacking a reference region, and is proving a useful tool in the application of imaging to drug development.

Conflict of interest

The authors declare no conflict of interest.

References


