Quantitative Comparison of [^{18}F]Fallypride PET Binding Potential Estimates Using Reference Tissue Models in Rat Brains

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***Abstract*– The purpose of our study was to compare commonly used mathematical models for analyzing dynamic [^{18}F]fallypride PET data in rat brains when arterial input function is not available. For the quantification of dopamine $D_{2/3}$ receptors, we compared the time activity curves (TACs) and binding potential estimates using several simplified methods, including the 1T simplified reference tissue model (SRTM), using 60 minutes versus 90 minutes of emission recording, the 2T Reference method, and the equilibrium concentration ratios. The reliability of fit of the tissue time activity curves (TACs) and the binding potential estimates were then compared.**

I. Introduction

Diverse methods for the quantification of dopamine $D_{2/3}$ receptors in primate and rat brains using ^{18}F-fallypride have been reported. In particular, the 1T simplified reference tissue model (SRTM) [1] has been widely used. The model's simplicity, however, and its ability to generate good fits to the data, even when the model assumptions are not met, can lead to improper dataset interpretation. Thus, we calculated and compared binding potential (BP_{ND}) values obtained from SRTM (single-tissue compartment) versus Watabe's reference tissue model (two-tissue compartment) using the cerebellum as reference for both methods [2]. It's also known that ^{18}F-fallypride has substantially higher affinity (33 pmol/L) to the D2/3 receptors than other benzamines, including ^{11}C-raclopride, making it more sensitive for the detection of extrastriatal $D_{2/3}$ receptors. However, the same high affinity necessitates lengthy scan times to obtain equilibrium binding estimates. In order to save time and increase efficiency of scanning, we tested whether SRTM analysis using 60 minutes of emission recording would still provide reliable results in comparison to 90 mins (60 vs. 90).

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II. Methods

A. ^{18}F-fallypride - PET

Twenty-three rodent brains were imaged with ^{18}F-fallypride (~ 1 nmol/kg) [3]. Animals were anesthetized with 2-2.5 % isoflurane air/oxygen mixture. The intra-subject variability of the depth of anesthesia was monitored by measuring respiratory frequency periodically during the scan. PET experiments were performed on a Bio PET/CT tomograph (Bioscan Inc., Washington, D.C.) with an axial field of view (FOV) of 4.8 cm and 6.7 cm in diameter. Time coincidence window was set to 10 ns with an energy window of 250-700 keV. The lateral tail vein was cannulated for injection of radiotracer, and the cannula was then connected to a heparin lock and secured in place with medical tape. The animal was positioned prone with the head placed symmetrically in the center FOV on the thermostatically heated bed supplied by the manufacturer (Bioscan Inc., Washington, D.C.). [^{18}F]fallypride injection of 1.06 ± 0.18 mCi (39.2 ± 6.7 MBq; 1.97 ± 1.05 nmol/kg. body weight) was then administered as a bolus injection (30 s) into the indwelling intravenous catheter followed by a 300 µl saline flush (maximum volume of injection= 600 µl). PET emission data was acquired for 90 minutes in list mode. The resultant emission sinograms for each frame were then corrected for scatter, ^{18}F decay, randoms, and deadtime. The datasets were reconstructed into a dynamic sequence of 14 individual frames (6 x 60 s, 1 x 300 s, 7 x 600 s) using FORE and OSEM-2D (16 subsets and 4 iterations) algorithm with a bin size of 0.39 mm, a matrix size of 175 x 175 x 61, and resolution recovery of 2.1 mm FWHM. Animal care and all experimental procedures were approved by the Animal Care and Use Committee (ACUC) of the National Institutes of Health (NIH).

B. Image analysis

The reconstructed PET images were spatially normalized into a standard space using an MRI atlas provided by Schweinhardt et al. [4] which is in stereotaxic space using a rigid body transformation matrix. The rat striatum was clearly visible bilaterally and delineated as previously described [3]. Volumes of interest (VOIs) were drawn over the ventral striatum (VS), thalamus (Th), and cerebellum (CB) using the co-registered MRI for guidance and an anatomical atlas of the rodent brain. Separate VOIs were drawn in the left and right

hemispheres and the values were then averaged. To minimize partial volume effects, VOIs were placed centrally within the structures. Image analyses were performed using PMOD 3.4 kinetic modeling tool (PMOD Technologies Ltd., Zurich, Switzerland). Time-activity curves (TACs) were generated for the VOIs.

C. Simplified reference tissue model

SRTM characterizes the kinetic behavior of a radioligand in both the target and reference region by a single-tissue compartment. There are four principal model assumptions: (1) no displaceable binding in the reference region, (2) tracer kinetics in the target and reference region can be represented by a single compartment model, (3) the blood volume differences to both the target and reference region are negligible, and (4) the target and reference tissue have the same nondisplaceable volume of distribution.

For each tissue, a differential equation that describes the kinetics of the radioligand as a function of the arterial plasma input function could be derived. Rearrangement and substitution of the equation into the target equation allows the elimination of the plasma term and yields the standard operational equation for SRTM,

$$C_T(t)=R_1C_R(t)+\left(k^2-\frac{R_1k_2}{1+BP_{ND}^{target}}\right)C_R(t)\otimes e^{-\frac{k2}{1+BP_{ND}^{target}}t} \quad (1)$$

where R1, is the relative target to reference rate of delivery (target to reference K_1 ratio), k_2 is the efflux rate constant, BP_{ND} is the binding potential to the target region and $C_T(t)$ and $C_R(t)$ are the time activity curves in the target and reference regions, respectively.

D. 2T Reference model

An extension of the SRTM assumes a displaceable signal in the reference tissue that can be expressed as two-tissue compartments (2T Reference) [2].

$$C_T(t) = R_1\left[C'(t) + aC'(t) \otimes e^{-(k_3'+k_4')t} + bC'(t)x \otimes e^{-k_2t}\right]$$

$$a = \frac{k_3'k_2'}{k_3'+k_4'-k_2}$$

$$a = \frac{k_2^2-k_2(k_2'+k_3'+k_4')+(k_2'k_4')}{k_3'+k_4'-k_2+} \quad (2)$$

$$BP_{ND} = \frac{K_1k_2'}{K_1'k_2\left(1+\frac{k_3'}{k_4'}\right)} - 1$$

When the parameters k_2', k_3', and k_4' are fixed to constants determined with the target tissue, only the R_1 and k_2 need to be fitted for BP_{ND} estimates. Reliable fits of the TACs and parameters estimates were evaluated using χ^2 test.

III. Results

A. Time-Activity curves

The TACs obtained from the ventral striatum (high binding region), thalamus (medium binding region), and the cerebellum (low binding region) are shown in Figure 1. Watabe's reference tissue model with two compartments exhibited the best quality of data fit in both the ventral striatum and thalamus. On the other hand, a modest over estimation and underestimation of fit was described with SRTM in the ventral striatum (early time points vs. later time points, respectively). No marked difference it quality of data fit was seen in the thalamus with either model.

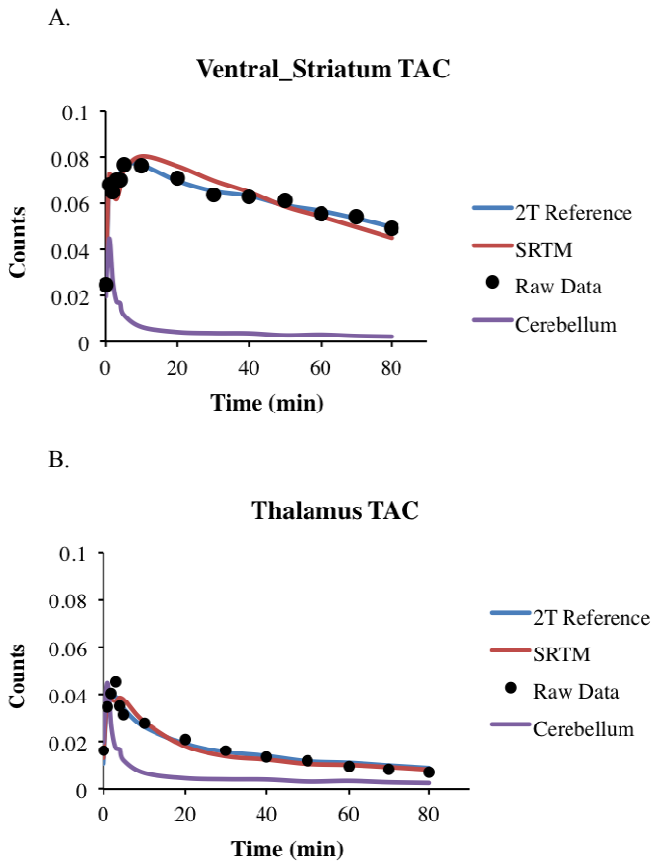

Fig. 1. TACs of ^{18}F-fallypride acquired for 90 min in an adult wild-type rat. Data shown are from the ventral striatum (A) and thalamus (B) with cerebellum as reference. The solid lines represent the quality of fit obtained using the 2-T Reference method (blue) and SRTM (red).

B. BP_{ND} estimates

Tables I & II show mean BP_{ND} and standard deviation (±SD) estimates using three simplified reference tissue methods. In comparing the methods, the coefficient of variation (COV), number of degrees of freedom (df), and χ^2 are provided as well.

TABLE I. BP_{ND} ESTIMATES IN THE STRIATUM (HIGH BINDING REGION)

KINETIC MODEL	BP_{ND}	±SD	COV	df	x^2
2T REF	14.78	1.53	10 %	9	0.98
SRTM (90)	13.51	1.57	12 %	11	2.83
SRTM (60)	13.05	2.20	17 %	8	3.19
EQ RATIO	16.25	2.20	14 %	–	–

TABLE II. BP_{ND} ESTIMATES IN THE THALAMUS (MEDIUM BINDING REGION)

KINETIC MODEL	BP_{ND}	±SD	COV	df	x^2
2T REF	2.06	0.19	10 %	9	12.09
SRTM (90)	2.07	0.18	8 %	11	7.91
SRTM (60)	2.17	0.22	10 %	8	6.90
EQ RATIO	2.04	0.21	10 %	–	–

In receptor rich striatal regions, the 2T Reference computations produced the best fit BP_{ND} estimates (χ^2 of 0.98) compared to conventional methods. Furthermore, the 2T Reference yielded higher (+20%) BP_{ND} estimates with lower (-17%) COV compared to SRTM (90). The largest variability was observed using SRTM with the shorter emission recording of 60 min. The simple ratio method substantially overestimated the BP_{ND} of ^{18}F-fallypride compared to the 2T Reference model. In the thalamus, however, the mean ^{18}F-fallypride BP_{ND} estimates were in close agreement irrespective of the method used.

IV. CONCLUSIONS

Reference tissue methods for calculation of ^{18}F-fallypride BP_{ND} in rat brains are suitable for VOI analysis when no plasma input is available. The choice of analysis method to derive the BP_{ND} can however influence the mean ^{18}F-fallypride BP_{ND} in high binding regions thus requiring lengthy scan times to obtain equilibrium binding estimates in the striatum. However, the effect of the analysis method in moderately binding regions was less noticeable.

In sum, the 2T Reference with 90 min scan time appeared as the method of choice due to its reliability, low variability and absence of bias (closer to a linear function). Trying to use SRTM method with only 60 minutes of emission data resulted in underestimation of the BP_{ND} values in high binding areas, along with greater variability. Acquiring the full 90 minutes of emission data thus appears necessary.

REFERENCES

[1] A. A. Lammertsma and S. P. Hume, "Simplified reference tissue model for PET receptor studies," *Neuroimage,* vol. 4, pp. 153-8, Dec 1996.

[2.] H. Watabe, M. A. Channing, M. G. Der, H. R. Adams, E. Jagoda, P. Herscovitch*, et al.*, "Kinetic analysis of the 5-HT2A ligand [11C]MDL 100,907," *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism,* vol. 20, pp. 899-909, Jun 2000.

[3] D. E. Lee, W. C. Reid, W. G. Ibrahim, K. L. Peterson, M. R. Lentz, D. Maric*, et al.*, "Imaging Dopaminergic Dysfunction as a Surrogate Marker of Neuropathology in a Small-Animal Model of HIV," *Mol Imaging,* vol. 13, pp. 1-10, Sep 1 2014.

[4] P. Schweinhardt, P. Fransson, L. Olson, C. Spenger, and J. L. Andersson, "A template for spatial normalisation of MR images of the rat brain," *J Neurosci Methods,* vol. 129, pp. 105-13, Oct 30 2003.