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RAPID COMMUNICATION

Initial Comparison of ntPET with Microdialysis Measurements of Methamphetamine-Induced Dopamine Release in Rats: Support for Estimation of Dopamine Curves from PET Data

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Abstract

A recently introduced mathematical method for extracting temporal characteristics of neurotransmitter release from dynamic positron emission tomography (PET) data was tested. The method was developed with the hope that by uncovering temporal information about neurotransmitter (nt) dynamics in PET data, researchers could shed new light on mechanisms of psychiatric diseases such as drug abuse and its treatment. In this study, we apply our model-based method, "ntPET", to ^{11}C-raclopride PET scans of rats in which the dopaminergic response to a microinfusion of methamphetamine in one striatum was assayed simultaneously by microdialysis and PET. Uptake of ^{11}C-raclopride into the untreated contralateral striatum was used as an input to the ntPET model. Direct comparisons of the model-based ntPET analysis and the microdialysis measurements confirmed that ntPET produced dopamine curves that were very similar in timing (takeoff and peak times) to the microdialysis curves. Variances in takeoff and peak times were comparable for the two methods. Neither method detected a false dopamine response to drug in a control animal. The high degree of correspondence between ntPET estimates and microdialysis measurements lends strong support to the idea that temporal information regarding dopamine release exists in dynamic ^{11}C-raclopride PET data and that it can be estimated reliably *via* ntPET. The method is entirely translatable to human PET imaging.

Key words: PET, Modeling, Drug abuse, Temporal response

Introduction

We recently introduced mathematical methods of estimating the time variation in stimulus-induced dopamine fluctuations in the striatum based on the analysis of paired bolus positron emission tomography (PET) studies [1–3]. We believe that these methods of analysis, collectively referred to as "ntPET" (neurotransmitter PET), could extend the use of PET and receptor ligands to explore temporal aspects of endogenous neurotransmitter release in human subjects. ntPET might be applied to investigate prominent hypotheses in addiction research such as the idea that the differential addictive liabilities of iv cocaine and oral methylphenidate relate to the respective rates of dopaminergic response that are elicited by each drug [4–7].

In effect, we are trying to develop ntPET into an imaging method akin to noninvasive microdialysis that could be used not only in people but longitudinally in experimental animals. It has been suggested that microdialysis is the gold standard by which we should gauge the success of our imaging-based

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methods. Microdialysis measures dopamine (DA) "directly", subject to certain assumptions; ntPET measures DA indirectly *via* a kinetic model of competition between DA and exogenous tracer. We defer an in-depth discussion of the precise relationship between the outcomes of the two assays; nevertheless, we present the first head-to-head comparison of the DA curves derived from each of the methods.

We note that there have been previous comparisons of microdialysis and PET or single photon emission computed tomography [8–11]. In none of the previous studies were the data analyzed in a way that could provide a direct comparison of DA curves. Early studies lead to a pair of observations of well-publicized and much-debated differences in the methods. First, the percent elevation in DA—as measured by microdialysis—after amphetamine administration was many times baseline, whereas the change in binding of ^{11}C-raclopride—as measured by PET—decreased by 20–30%. Second, the deflection of the raclopride curve appeared to persist for longer than the microdialysis measurements of DA remained above baseline. Follow-up studies with various doses and species have shown a return of raclopride binding to pre-amphetamine levels in 8–24 hours [12, 13] with possible long-term changes in affinity of the tracer for the receptor [14]. The first observation above is merely a reflection of the fact that DA concentration is virtually unlimited, whereas change in binding is an inherently limited quantity, as binding sites are limited. The second observation may mean that displacement of dopamine-receptor tracers by some drugs does not adhere to a purely competitive model [15, 16]. The modeling method that we test here assumes a purely competitive system of DA and raclopride at a single receptor site.

Microdialysis and ^{11}C-raclopride PET imaging were performed simultaneously on rats that received an infusion of methamphetamine intracerebrally into the striatum, unilaterally. The data were originally acquired as part of an ongoing effort to combine the two experimental methods to study the effect of stimulants on DA in animals [10, 11]. Recently, the data were made available (to EDM, MDN), and ntPET analyses to estimate time variation in DA from the PET data were performed retrospectively. Although the experiments were not ideally designed for use with ntPET, the results provide initial support for the emerging method as well as some insight into its possible limitations.

Methods

This work was approved by the BNL IACUC. Adult male Sprague–Dawley (Taconic Farms, NewYork) rodents weighing 250–489 g were used in the present analysis. All animals were housed and maintained in an AAALAC accredited veterinary facility.

Microdialysis

Surgery and Preparation Microdialysis surgery was performed as described previously [17]. Briefly, 2 days before microPET imaging, siliconized guide cannulae were surgically implanted above the corpus striatum according to coordinates established with the atlas of [18]; 0.5 mm anterior and 1.5 mm lateral to bregma, 2.5 mm below the surface of the brain. Dental acrylic and surgical screws were used to fix the position of each guide cannula. On the day of the PET experiment, animals were first placed into microdialysis chambers [RatTurn®, Bioanalytical Systems, Inc. (BAS), West Lafayette, IN] where concentric flow dialysis probes (340-μm OD×4-mm length, BAS) were inserted into the guide cannulae in awake animals. Microdialysis probes were connected to a microinfusion syringe pump with small bore tubing (120-μm ID, 0.5-m length), and animals were tethered to a rotating connector to prevent tangling before anesthesia for scanning. The dialysis probes were perfused with artificial cerebrospinal fluid (aCSF; 155 mmol NA^+, 1.1 mmol Ca^{2+}, 2.9 mmol K^+, 132.76 mmol Cl^-, and 0.83 mmol Mg^{2+}) at a flow rate of 2.0 μl/min. After at least 60 minutes of equilibration and approximately 40 minutes before scheduled injection of radioligand, animals were anesthetized with an intraperitoneal injection of ketamine/xylazine (100 and 10 mg/kg) and positioned in the tomograph gantry using a nylon stereotactic head holder (Kopf Instruments, Tujunga, CA, USA). Once positioned in the gantry, animals were reconnected to the microinfusion pump and also to a refrigerated fraction collector that was preset to a temperature of 4°C (BAS). To allow for administration of radioligand, a lateral tail vein was catheterized using a 24-gauge catheter. Anesthesia was maintained during the imaging session (2–4 hours) by intramuscular (i.m.) injections of ketamine/xylazine at a dose averaging 85 mg kg^{-1} $hour^{-1}$.

Sample Collection and Analysis Samples were collected into capped sealed vials every 3.0 minutes and injected onto microbore high-pressure liquid chromatography (HPLC) systems using an automated device (Sample Sentinel, Bioanalytical Systems, Inc.). Details of the microbore HPLC and electrochemical detection procedure (mobile phase contents, pump details, flow rates, and electrode characteristics) can be found elsewhere [19]. The relative recovery of DA from the striatum was estimated, as described previously [10], to be 20%. Microdialysis data were calibrated using *in vitro* standards and a calculated extraction fraction [20] to yield nanomolar concentrations, in addition to being normalized to the three baseline samples that varied by less than 10% before ^{11}C-raclopride injection.

PET Imaging

Nine (9) animals in the original cohort received microPET scans after bolus injection of ^{11}C-raclopride. In eight (8) of nine animals, methamphetamine (10^{-6} M) was administered *via* unilateral striatal microinfusion at some point after ^{11}C-raclopride. ^{11}C-Raclopride was synthesized according to [21]. SA determinations were made using mass measurements acquired during radiotracer purification by HPLC (Waters Novapak C18; 250×10 mm) and radioactivity measurements obtained with a calibrated ion chamber (Capintec, Inc., Ramsey, NJ). At the time of injection, SA ranged from 0.167 to 1.34 Ci/μmol. The injected activity of ^{11}C-raclopride ranged from 184 to 798 μCi corresponding to a mass dose of 0.37–13.1 nmol/kg body weight. MicroPET imaging was performed using an R4 tomograph (Concorde Microsystems, Knoxville, TN), which has a 12-cm animal port with an image field of view of ~10 cm. Each animal was positioned with its head in the center of the field of view. Emission scans began simultaneously with intravenous radioligand administration into the tail vein and

continued for between 65 and 90 minutes. At the center of the field of view, the R4 has a transaxial resolution of approximately 1.85 mm full-width at half-maximum (FWHM) and remains less than 2.5 mm FWHM across the rat brain [22].

PET data were binned into a series of graduated time frames (a typical series covering 60 minutes was: 2×60 seconds; 4×120 seconds; 10×300) and included subtraction of random coincidences collected in a delayed time window. The resulting sinogram data were rebinned using Fourier rebinning and reconstructed with two-dimensional filtered backprojection (FBP) with a ramp filter (Nyquist cutoff) and software provided by the manufacturer. Image pixel size in FBP reconstructed images was 0.85 mm transaxially with a 1.21-mm slice thickness

Post-Processing of Microdialysis Curves

Microdialysis curves for five animals that received drug were fitted to gamma-variate curves that included a variable takeoff time, t_D. As the sample concentrations are reported as fraction of baseline, the curves were fitted to:

$$\mathrm{DA}_{\mu-\mathrm{dial}}(\mathrm{t})/\mathrm{Basal} = 1 + \frac{\gamma(t-t_D)^{\alpha}e^{-\beta(t-t_D)}}{\mathrm{Basal}} \qquad (1)$$

where Basal is the baseline concentration of DA as measured by microdialysis before drug, $[DA_{\mu-dial}]$. The peak time of the function in Eq. 1 is always α/β. The normalized peak heights reported below are calculated as $[\mathrm{DA}_{\mu-\mathrm{dial}}(\alpha/\beta)]/\mathrm{Basal}$.

Extra care was taken to properly place the microdialysis samples and drug arrival time onto the PET timeline to ensure the accuracy and validity of our comparison. The microdialysis samples were each plotted at the midpoint in time of the collection period for each sample (3 minutes long). The transit time of each sample to traverse the lines from the brain to the sample collector was subtracted from the microdialysis times. The drug administration times were incremented by the transit time from the syringe to the brain to get the time at which the drug arrived at the brain.

Data Exclusion Criteria

In total, eight animals in the cohort that we attempted to analyze with ntPET received an infusion of methamphetamine intracerebrally through a microdialysis probe. Because our goal was to compare microdialysis to ntPET quantitatively, we excluded data sets for the following reasons: (1) The $[DA_{\mu-dial}]$ curve took off from baseline before the drug was administered (one case). (2) Microdialysis data were incomplete; samples were taken too infrequently or not late enough to capture the peak (i.e., at least one descending point) of the $[DA_{\mu-dial}]$ curve after the start of drug infusion (two cases). (3) *Both* methods produced an estimated peak time that—when considered relative to the mean of the other estimates by the same method—was an outlier (one case). One of the excluded data sets is presented in "Results".

ntPET Analysis

We have recently introduced two different approaches to estimate the DA curve from PET data [1, 2]. Both methods require two sets of PET data: one from the resting state, the other from the activation state (i.e., when drug is administered). The first approach is based on a compartmental model [2, 3, 23], whereas the second is independent of any model and uses singular value decomposition to find (linear) components in the PET data that are unique to the activation state. Only the first method was used in the present retrospective study.

Model-based ntPET fits a compartmental model to two PET time–activity curves (one each from rest and activation scans) at the same time. Because the rats in this study were treated unilaterally with methamphetamine, there was only one scan session. As the drug was delivered directly to the right striatum, the time–activity curve (TAC) from the contralateral striatum was used as the "rest" curve and the TAC from the ipsilateral side (receiving drug) was used as the "activation state" curve. Because no arterial samples were taken, the REF variant of model-based ntPET was used [3]; input functions were derived from cerebellum curves in a manner similar to the earliest reference tissue models [24, 25]. The model assumes that the DA effect on the PET data follows a gamma-variate functional form. Thus, by fitting the model to the PET data, we estimate the standard parameters that describe uptake of the tracer and the parameters of the function that describes DA. For clarity, we note that the microdialysis data were also fitted to a gamma-variate (see Eq. 1). That prost-processing step was entirely independent and separate from our analysis of the PET data. It was implemented to foster the most straightforward comparison with the ntPET results.

The mass balance equation that is added to the conventional two-tissue compartment model describes the change in concentration with time of bound DA,

$$\frac{dB^{\mathrm{DA}}(t)}{dt} = k_{\mathrm{on}}^{\mathrm{DA}}\left[B_{\max} - B(t) - B^{\mathrm{DA}}(t)\right]DA_{\mathrm{ntPET}}(t) - k_{\mathrm{off}}^{\mathrm{DA}}B^{\mathrm{DA}}(t) \qquad (2)$$

where $B_{\max}$ is total receptors, and $k_{\mathrm{on}}^{\mathrm{DA}}$ and $k_{\mathrm{off}}^{\mathrm{DA}}$ are the association and dissociation rate constants, respectively for DA. $B(t)$ is bound tracer and $\mathrm{DA}_{\mathrm{ntPET}}(t)$ is the time-varying free DA concentration (to be estimated *via* ntPET). $B_{\max}$ is fixed in our analysis. To improve the identifiability of the fits, we constrained the fitting (cost) function using relevant prior information. One penalty function, described at length in [2], was used to penalize the individual parameters so that estimated binding potential (BP) would be close to an independently acquired measure of BP ($\mathrm{BP}^{\mathrm{meas}}$) in the resting state [26]. Priors were applied to penalize peak time estimates if they were earlier than the time of methamphetamine administration, t_{meth}, by microinfusion. For practical reasons of data fitting, the peak time estimates later than the end of the PET acquisition, t_{end}, were also penalized. t_{end} varied across animals. The cost function can be written as,

$$\begin{aligned}\Phi(\Theta_{\mathrm{TR}},\Theta_{\mathrm{DA}}) &= \sum_j\sum_i w_{ij}\left[PET_{ij}^{\mathrm{meas}} - PET_{ij}(\Theta_{\mathrm{TR}},\Theta_{\mathrm{DA}})\right]^2 \\ &+\tau_1\left[\exp\left(-\left[t_{\mathrm{p}}(\Theta_{\mathrm{DA}}) - t_{\mathrm{meth}}\right]\right) + \exp\left(t_p(\Theta_{\mathrm{DA}}) - t_{\mathrm{end}}\right)\right] \\ &+\tau_2\left[BP^{\mathrm{meas}} - BP(\Theta_{\mathrm{TR}},\Theta_{\mathrm{DA}})\right]^2\end{aligned} \qquad (3)$$

where j is the index over data conditions (i.e., with or without drug), i is the index over data points in each session, w_{ij} are the weights applied to each residual (based on the variance of data point i), and τ_1 and τ_2 are weights applied to the penalty terms ($\tau_1=\tau_2=60$). Θ_{TR} and Θ_{DA} are the vectors of tracer and DA parameters, respectively. To combat against converging to local minima in the cost function, we fitted each data set with 50 different random, and quite varied, initial parameter guesses [3]. The final output is a collection of the best fits to the data as determined by an objective method using the residual sum of squares and the number of "runs" in the residuals. The method has been described in detail elsewhere [2, 3].

Results

Figure 1 shows various views of an axial slice through the striatum of an animal who was scanned with ^{11}C-raclopride while samples were collected from an in-dwelling microdialysis probe in the striatum. The top row shows a slice from an MR template [27] at the same anatomic location as the position of the probe. The PET data on left is an average of the 20- to 60-minute raclopride frames. The figure on the right is an image of the probe, perfused with 2-deoxy-2-[F-18] (FDG), in the same animal. The bottom rows show various overlays. Notice that right striatum (which received methamphetamine through the probe) shows less tracer uptake than left striatum (no drug).

Figure 2 shows the direct comparison between microdialysis measurements of the DA curve (filled circles) fitted to a gamma-variate curve (red, dashed) and the family of gamma-variate functions (blue) that are estimated from the PET data using ntPET. The family of blue curves is produced by the best fits to the PET data as described previously [2]. Although the best fits are not identical, they are very nearly coherent, temporally. Note the close overlap of the family of ntPET-generated DA curves in time with the microdialysis curve. ^{11}C-raclopride was injected at time 0. Drug was infused through the probe beginning at 15 minutes post-tracer (vertical arrow) and arrived at the brain 2.8 minutes later.

Figure 3 shows an animal by animal comparison of estimated DA takeoff times (blue lines) and DA peak times (red lines) for the four data sets that met our inclusion criteria. The mean takeoff time by microdialysis was 18.3±6.8 minutes after drug reached the brain. By ntPET it was 19.9±5.0 minutes after drug. The mean peak time was estimated by microdialysis to be 34.8±7.1 minutes, whereas ntPET estimated the mean DA peak time to be 33.1±10.6 minutes after methamphetamine infusion began. Neither takeoff times nor peak times were significantly different across methods, but the samples were admittedly small (n=4).

Figure 4 shows the same type of result as in Fig. 1 for an animal that did not receive any methamphetamine. The microdialysis trace shows only a slight response of DA to raclopride (injected mass dose was 3.9 nmol/kg); see [10] for a detailed examination of this issue. Similar small responses of DA to raclopride are common to all microdialysis traces, but this response is not apparent in Fig. 2 because of the scale. The ntPET model finds no DA response whatsoever (flat blue line). The lower penalty on peak time, t_{meth}, was set to 0 for this animal in the absence of any prior information about drug administration.

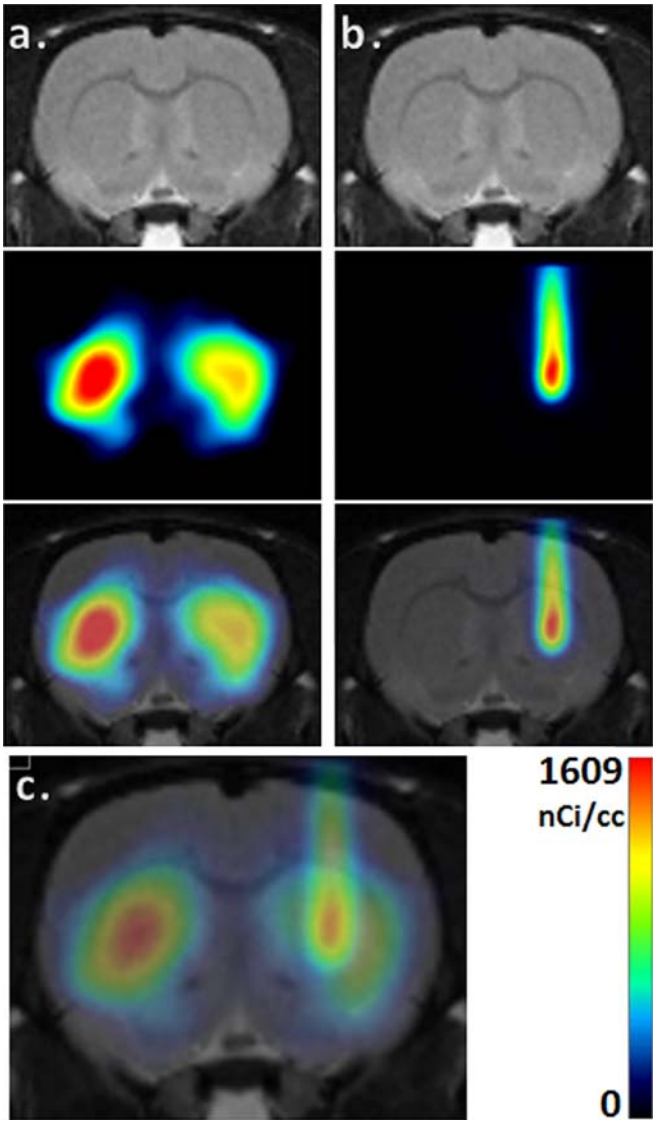

Fig. 1. Infusion of methamphetamine through the microdialysis probe increases extracellular DA and displaces ^{11}C-raclopride. **a** *Maximum a posteriori* (MAP) reconstructed ^{11}C-raclopride radioactivity distribution is shown from a summed PET acquisition where methamphetamine was infused in the right striatum through a microdialysis probe. **b** Probe verification in three dimensional space is shown by microinfusion of FDG (~30 μCi) immediately after the ^{11}C-raclopride experiment. The two images are overlaid in **c**. Images were spatially preprocessed and normalized to a stereotaxic atlas of the rat brain [27] using the SPM2 software package.

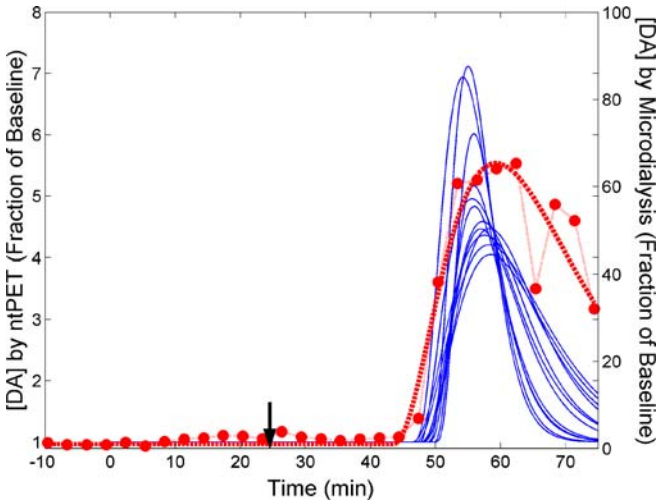

Fig. 2. Family of best fits to the PET data (*blue*) compared to smooth function fit directly to the microdialysis data (*red curve* through *red circles*). Temporal agreement is quite good.

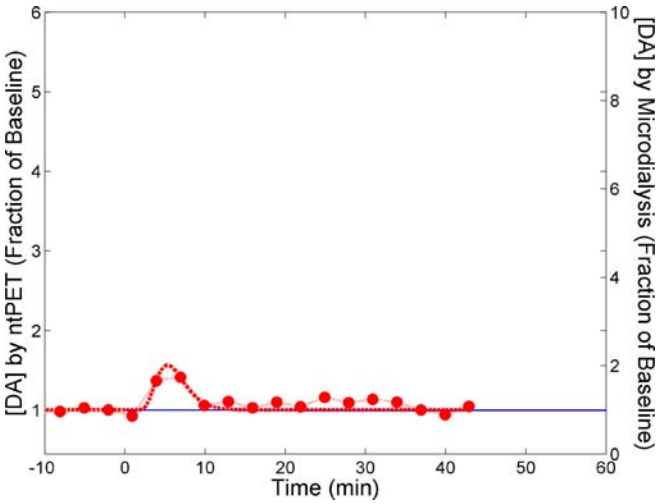

Fig. 4. Analysis of null data. No drug given. ntPET found no DA response. Microdialysis found only the initial DA response to raclopride but no *false* responses found.

Figure 5 presents a microdialysis vs ntPET comparison for an animal which was ultimately excluded (see exclusion criteria in "Methods"). The microdialysis curves (red, dashed) take off from baseline only a few minutes before the estimated takeoff *via* ntPET, suggesting agreement. Although the data had to be excluded because the microdialysis curve was still increasing at the end of the microdialysis sampling (so a peak time could not be calculated) the correspondence between the fitted $DA_{\mu-\mathrm{dial}}(t)$ and the estimate $\mathrm{DA}_{\mathrm{ntPET}}$ is clearly high. Inset shows same comparison on a log scale.

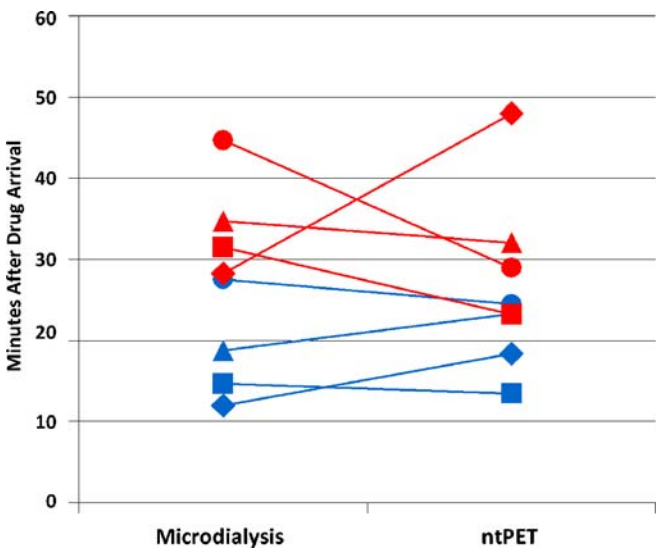

Fig. 3. Point by point comparison between microdialysis and ntPET. *Blue lines* connect estimates of takeoff time; *red lines* connect estimates of peak time. A single symbol (*squares*, *triangles*, *diamonds*, and *circles*) is used for each animal.

Discussion

Correspondence and Variability of Methods

In examining four restrospective rat studies quantitatively, we find considerable agreement between ntPET estimates of the timing of DA release and the microdialysis measurements. An additional study, shown in Fig. 5, was excluded from our quantitative analysis, but the results of the two methods are clearly in agreement. The means and variances in takeoff times were similar (slightly favoring ntPET), but the variability in ntPET estimates of peak time was slightly

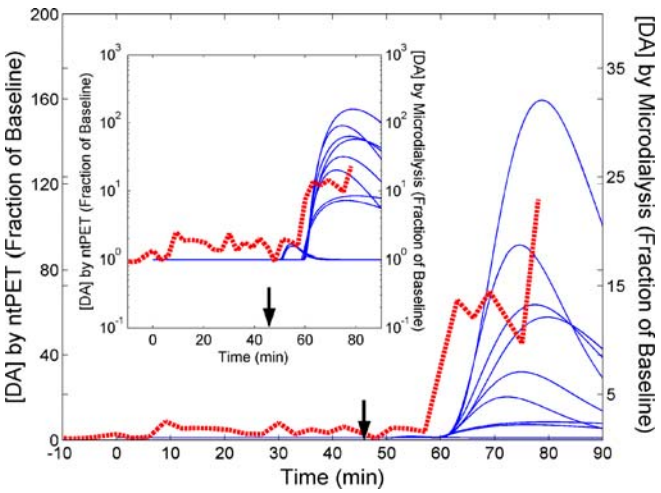

Fig. 5. Comparison of family of curves generated by ntPET (*blue*) vs microdialysis (*dashed red*) for an animal that ultimately had to be excluded from quantitative analysis in Fig. 3. We were unable to determine a peak time from microdialysis (see "Data Exclusion Criteria") but visually, the microdialysis curve resembles the ntPET. Same comparison on log(DA) scale is *inset*.

worse than for microdialysis. We attribute any underperformance of the ntPET method to four possible causes. (1) The peaks in the DA curves occur near the end of the PET acquisition. Theoretical work [3] demonstrates that variability of peak time estimates degrades as the release of DA tends toward the end of the PET scan. This is not surprising. Late in the scan, the signal to noise ratio in the PET data is low, and the likelihood of the ntPET model mistaking noise for an effect of DA is increased. Longer frame times will also degrade the temporal resolution of our method. (2) The data in this study were not ideally suited to the ntPET method which assumes a unimodal, gamma-variate-like response of DA, the type of response one would expect to a bolus injection of drug rather than an infusion. (3) Placement of the probe is variable and may not deliver drug to all parts of the striatum uniformly. Microdialysis samples were drawn from tissue closest to the probe—the same tissue that was most exposed to the infused drug. On the other hand, PET data were derived from a region of interest (ROI) placed on whole striatum which probably included tissue that was not uniformly perfused by the aCSF that contained the drug. (4) ntPET assumes that the resting state uptake of tracer and the activation state uptake of tracer are identical (same tracer parameters, Θ_{TR}) save the effect of DA. Normally, the data would be acquired in a two-scan protocol, and data from an ROI at rest would be analyzed along with data from the same ROI during activation. In this study, the left side was used for "rest" and the right side (got drug and) was used for "activation". But left and right striata—especially in the presence of a microdialysis probe implant—may not be the same.

Null Data

We find it quite encouraging that the ntPET fit of data shown in Fig. 3 found no DA response, which was in accord with the microdialysis trace. As there was no significant difference between the injected activity in this "null" experiment (402 μCi) and the activity in the other experiments (463±224 μCi), we would expect the same level of noise, and thus, the same likelihood of a false-positive detection of DA release. The fact that there was none gives us additional confidence in the ntPET DA curve estimates for the four animals included in Fig. 3.

Calibration of the DA Curves

We have not focused on the estimates of the amplitude of the DA curves by either method. Microdialysis measures "extracellular" dopamine from a sample volume of tissue immediately surrounding an inserted probe. The efficiency of the probe and the recovery of dopamine from the extracellular space are functions of the microdialysis membrane as well as the state of the tissue in the sample volume. If the state of the sampling environment changes (e.g., by an alteration in dopamine uptake caused by methamphetamine), the efficiency of the probe may change and the scale of the microdialysis DA measurements becomes unknown [20, 28]. Thus, there is some uncertainty associated with the amplitude of DA assayed by conventional microdialysis. Because of correlation between parameters that describe the scale of the DA curve in the ntPET model and other model parameters that describe the uptake of tracer, we have shown previously [3] that the normalized height of the DA curves is not as identifiable as its shape (i.e., temporal aspects).

The data presented here, while not an ideal test of ntPET, represented a unique resource. Thus, we chose to examine them retrospectively with the understanding that the validity of our new method would not be established, unequivocally. The observed correspondence between ntPET and microdialysis—as well as recognition that they do not measure identical phenomena—gives us encouragement to continue to develop our methods. A limitation of the present model-based method may be that it is designed for a DA response of a particular functional form. Parallel efforts in our group have lead to a second ntPET method that makes no assumptions about the shape of the DA curve. New experiments are currently underway to further validate and characterize both ntPET approaches.

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