DEVELOPMENT OF A DUAL TRACER PET METHOD FOR IMAGING DOPAMINERGIC NEUROMODULATION

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The modulatory neurotransmittor dopamine (DA) is involved in movement and reward behaviors, and malfunctions in the dopamine system are implicated in a variety of prevalent and debilitating pathologies including Parkinson's disease, attention deficit/hyperactivity disorder, schizophrenia, and addiction. Positron emission tomography (PET) has been used to separately measure changes in DA receptor occupancy and blood flow in response to various interventions. Here we describe a dual tracer PET method to simultaneously measure both responses with the aim of comparing DA release in particular areas of the brain and associated alterations in neural activity throughout the brain. Significant correlations between reductions in DA receptor occupancy and blood flow alterations would be potential signs of dopaminergic modulation, *i.e.* modifications in signal processing due to increased levels of extracellular DA. Methodological development has begun with rats undergoing an amphetamine challenge while being scanned with the blood flow tracer [^{17}F]fluoromethane and the dopamine D_2 receptor tracer [^{18}F]desmethoxyfallypride.

1. Dopaminergic Neuromodulation

Dopaminergic neuromodulation is the process in which dopamine released into the extracellular space modifies neurotransmssion at downstream synapses. It plays an important role in brain function and its malfunction is implicated in a number of pathologies.

1.1. *The Dopamine System*

Dopamine (DA) is one of several modulatory neurotransmitters in the brain along with serotonin, norepinephrine, acetylcholine, and others. Dopamine neuron cell bodies are concentrated in two midbrain regions, the substantia nigra (SN) and the ventral tegmental area (VTA). In the nigrostriatal circuit, cells in SN project to striatum, and in the mesolimbic circuit, cells in VTA project to

limbic areas including nucleus accumbens (NAcc) and prefrontal cortex (PFC). The nigrostriatal circuit is involved in movement while the mesolimbic circuit is associated with reward processing and attention.

1.2. *Modulation of Neurotransmission*

The majority of signalling events in the brain involve point to point activation of fast exitatory or inhibitory ionotropic receptors by glutamate or GABA. These synaptic connections may be suppressed or indirectly enhanced by the release over relatively large volumes of modulatory neurotransmitters such as dopamine, which act on slow presynaptic metabotropic receptors[1]. These processes are studied in animals with invasive procedures including stimulation and recording electrodes to follow electrical activity, as well as forward and reverse microdialysis to observe extracellular concentrations of neurotransmitters or to inject various agonists and antagonists.

2. Simultaneous Measures

To understand dopaminergic neuromodulation at the systems level in the brain, it is necessary to observe both the release of dopamine and related alterations in neural activity. Even with precise control of the experimental conditions, there will be some variability in the physiological state of the brain between measurements, and it is therefore best to make these observations simultaneously. This is especially true when studying the neural basis of human behavior where within subject variability is expected to be larger than in anesthetized animals. Except for rare cases where invasive techniques are practical in neurological patients, an imaging method is required that permits simultaneous measures of dopamine release and neural activity. Radiotracer techniques can be used to observe dopamine release by measuring the change in binding of receptor ligands due to competition from the endogenous neurotransmitter, in this case dopamine[2]. There are a number of possibilities for extending these PET and SPECT methods to obtain simultaneous measures of dopamine release and the related changes in brain activity.

2.1. *PET/EEG*

One possibility for studying dopaminergic neuromodulation is the combination of PET and electroencephalography (EEG). This technique was explored as early as 1984[3]. Published studies involve EEG measurement duing uptake of FDG outside the scanner with subsequent PET scanning, which reflects glucose

metabolism during the uptake period[4]. It might be feasible to perform EEG within the PET scanner while observing a dopamine receptor tracer. EEG provides good temporal resolution, however it suffers from poor spatial resolution in subcortical regions.

2.2. *PET/MRI*

Combining PET with magnetic resonance imaging (MRI) would also permit simultaneous measurement of dopamine release and neural activity as indicated by changes in the blood oxygen level dependent (BOLD) signal. A small PET ring has been successfully operated inside an MR scanner with light from the scintillators carried by fiber optics to photomultiplier tubes placed at a distance from the magnetic field of the MR[5]. Work is also underway to read out the scintillators with avalanche photodiodes (APD), which are insensitive to the magnetic field[6].

2.3. *Dual Tracer SPECT*

In single photon nuclear imaging techniques, tracers may be distinguished based on the energy of the emitted gamma. This was demonstrated in 1967 by simultaneously scanning patients with different combinations of tracers, for example visualizing lung with ^{131}I albumin macroaggregate and liver with ^{198}Au colloidal gold[7]. Recent work with simulations and phantoms suggests the feasibility of using dual tracer single photon emission computed tomography (SPECT) for simultaneous imaging of dopamine receptor binding and blood flow[8].

3. Dual Tracer PET

The present work involves a dual tracer PET method. In PET the annihilation photons are essentially monoenergetic at 511 keV regardless of the radioisotope, so there is no information directly available to the scanner that permits tracers to be distinguished from one another. However, it is possible to use the measured time course to distinguish tracers based on their physical half lives and their pharmacokinetics.

3.1. *Distinguishing Half Life*

In 1982 Huang *et al.* demonstrated with phantoms that ^{13}N ($t_{1/2}$ = 10 min) and ^{18}F ($t_{1/2}$ = 110 min) could be distinguished using PET based on their half lives[9]. In this case it was possible to parameterize the sinograms with a sum of exponentials and reconstruct the ^{18}F and ^{13}N images separately. However, because this technique requires the tracer spatial distribution to be constant over time it would not work in vivo except in limited cases.

3.2. *Distinguishing Pharmacokinetics*

Because of different uptake and clearance rates for the tagged molecules, it is possible to distinguish tracers. Koeppe *et al.* demonstrated this concept in humans with staggered paired injections of the benzodiazepine receptor agonist [^{11}C]flumazenil (FMZ), the acetylcholinesterase substrate N-[^{11}C]methylpiperidinyl propionate (PMP), and the vesicular monamine transporter ligand [^{11}C]dihydrotetrabenazine (DTBZ)[10]. With injections staggered by as little as 10-20 minutes, it was possible to measure relevant pharmacokinetic parameters with accuracy similar to that obtained in single injection scans for FMZ:DTBZ and DTBZ:FMZ as well as FMZ:PMP and PMP:FMZ.

3.3. *Modulated Delivery*

As a proof of principle for the simultaneous dual tracer technique described here, we performed baseline studies in rhesus monkeys[11]. Two monkeys were anesthetized with isoflurane, and positioned in the UW ECAT 933/04 tomograph with 6 mm FWHM resolution (CTI, Knoxville, TN). [^{18}F]fallypride ($t_{1/2}$ = 110 min), a dopamine D2 receptor antagonist, was injected (5.1 and 2.1 mCi). Starting 99 - 137 min after injection, 10 second PET images were acquired while the blood flow tracer [^{17}F]fluoromethane ($t_{1/2}$ = 65 s) was administered by inhalation (0.46 mCi/s in 2 mL/s neon carrier) in a repeating boxcar pattern of 45 s on / 45 s off for twelve minutes ("modulated delivery"). Images were reconstructed, and eight 2.0 mL regions of interest (ROI) were specified.

The observed 12 minute time-activity curves were split into 6 minute segments and independently fit with a three compartment lung - body - brain model of fluoromethane kinetics with whole brain perfusion fixed (Fig. 1). Repeatability of the dual tracer measures between one pair of consecutive 6 min scans is shown in Fig. 2. Averaging over both monkeys and all scans, the mean absolute variation in rCBF between consecutive 6 min scans in all 8 ROIs was 8.9% ± 7.3% (SD, n=32). The mean absolute variation in [^{18}F]fallypride concentration in the 2 striatal ROIs between consecutive scans was 7.9% ± 7.4% (SD, n=8). Sensitivity of measured rCBF to 20% variation in each of the assumed model parameters was shown to be <0.3%, except for the fixed value of whole brain perfusion, which was effectively a scale factor.

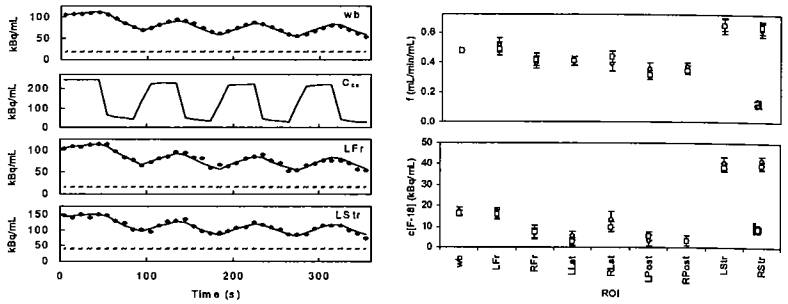

Figure 1. (left) Representative TACs from a 6-minute scan segment (10 second resolution) during modulated delivery of [^{17}F]fluoromethane, 117 min post-injection of [^{18}F]fallypride. (wb) whole brain TAC. (c_{ca}) modeled [^{17}F]fluoromethane cerebral arterial input function from fit to wb TAC. (LFr, LStr) TACs for 2 mL ROIs fit using $c_{ca}(t)$. ♦ observed radioactivity. — modeled total radioactivity. --- modeled [^{18}F]fallypride radioactivity.

Figure 2. (right) Observed rCBF (a) and [^{18}F]fallypride concentration (b) from two consecutive 6-minute scans during modulated delivery of [^{17}F]fluoromethane, ◇ 117 min and □ 123 min post-injection of [^{18}F]fallypride. Whole brain perfusion fixed at 0.48 mL/min/mL.

While these results suggested that the concept is feasible, it should be noted that the methods described below differ in a number of respects: (1) Blood sampling will be used to better determine the tracer input functions; (2) a pharmacokinetic model of the neurochemical tracer will be applied; (3) separate boluses of the blood flow tracer will be given making the analysis more precise and reducing the radiation dose to the subject.

4. Methods

The goal of the current work is to optimize a dual tracer PET protocol for measuring dopaminergic neuromodulation. Exploratory/developmental work is being carried out in anesthetized animals responding to a pharmacological challenge. A short lived blood flow tracer is administereed in the presence of a long lived dopamine receptor ligand. The measures of interest will be ΔrCBF, the change in blood flow, and ΔBP, the change in receptor binding.

4.1. *Subjects*

Sprague Dawley male rats (n=8, weight = 380 ± 42g, mean ± SD) were anesthetized with isoflurane (1.5%), and 24g catheters were placed in lateral tail veins for i.v. injection of radiotracers and amphetamine. Four animals at a time were positioned in the scanner with their heads fixed by ear and tooth bars.

4.2. *Scanner*

Scanning sessions were performed on the UW Concorde microPET P4 with 8 cm axial x 19 cm transaxial FOV, 2x2x2 mm^3 reconstructed image resolution, and 2% sensitivity at center[12]. Following a 10 min transmission scan, emission data were collected for 150 minutes in 3D list mode.

4.3. *Radiotracers*

The blood flow tracer [^{17}F]fluoromethane (FM) for injection was prepared using an in-line gas phase method developed in our lab[13]. The intermediate affinity dopamine D2 receptor ligand [^{18}F]desmethoxyfallypride (DMFP), (S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-F-18-fluoropropyl)-2-methoxy benzamide, was produced using published methods[14].

4.4. *Pharmacokinetic Model*

A dual tracer pharmacokinetic model will be employed to analyze the data (Fig. 3). We combine a two compartment ligand model with a single compartment flow tracer model, each of which is based on standard methods[15]. For a given ROI, blood flow and DMFP binding are assumed to respond instantaneously to a stimulus (amphetamine), *i.e.*, $k^f_1 \rightarrow k^f_1 + \Delta k^f_1$ ($k^f_2 = k^f_1$) and $k^l_3 \rightarrow k^l_3 - \Delta k^l_3$. Modelled TACs are generated iteratively on a 1 s grid.

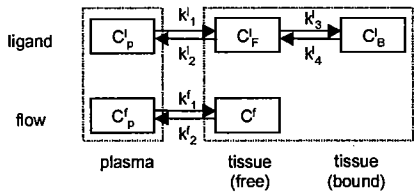

Figure 3. Dual tracer pharmacokinetic model showing compartments for ligand and flow tracer in plasma (c^l_p and c^f_p), free and bound ligand in tissue (c^l_F and c^l_B), and flow tracer in tissue (c^f).

Preliminary simulations of TACs generated using this model are shown in Fig. 4. In these simulations, an approximate [^{11}C]raclopride infusion input function is assumed, $c^l_p(t) = e^{-\lambda l t}$, where λ^l is the ^{11}C radioactive decay rate, ln(2)/20.4 min. Input functions for bolus injections of [^{17}F]fluoromethane at 900, 1800, 3600, and 4500 s are simulated with $c^f_p(t') = e^{-\lambda f t'} \times e^{-\lambda e t'}$, where t' is the time relative to the fluoromethane injection, λ^f is the ^{17}F radioactive decay rate, ln(2)/64.5 s, and λ^e is an approximate excretion rate, ln(2)/60 s. In the

simulation, rate constants were taken from the literature for human striatum: $k^1_1 = 0.15\ min^{-1}$, $k^1_2 = 0.37\ min^{-1}$, $k^1_3 = 0.51\ min^{-1}$, and $k^1_4 = 0.14\ min^{-1}$ [16].

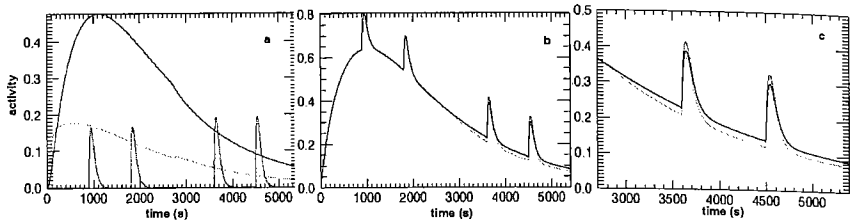

Figure 4. Compartmental model simulations of a dual tracer scan. **a** Contributions to the observed TAC from the flow tracer, bound ligand, and free ligand, with 20% reduction in ligand binding and 20% increase in blood flow at 2700 s. **b** Simulation of observed TAC with no response, 20% increase in blood flow, or 20% decrease in binding. **c** detail of post-stimulus observed TAC (2700-5400s).

5. Preliminary Results

First results were obtained in October 2005 with combined FM and DMFP imaging of rats during a d-amphetamine (AMPH) challenge (0.4 mg/kg i.v.). Eight rats were scanned with various combinations of FM x DMFP x AMPH. The PET images was coregistered with template ROIs based on histology[17]. The resulting TACs for one subject are shown in Fig. 5.

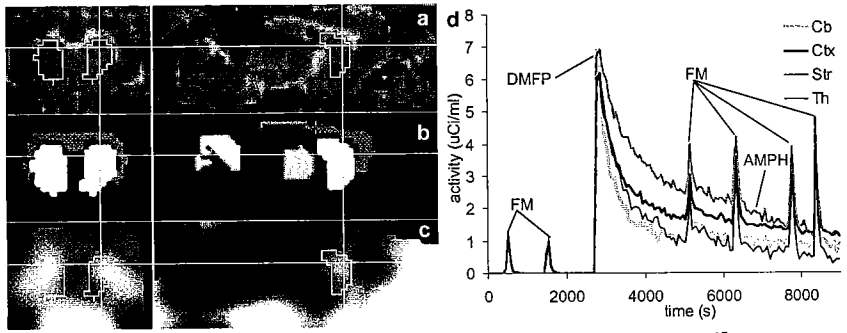

Figure 5. Dual tracer scan of a rat with [^{18}F]desmethoxyfallypride (DMFP) and [^{17}F]fluoromethane (FM). Coronal and sagittal views of (a) brain atlas, (b) regions of interest, and (c) PET image (2000 - 9000 s) with striatal ROI outlined. (d) Corresponding time activity curves at 60 s resolution. The bolus injection of DMFP (1.1 mCi) is seen at 2700 s. Six bolus injections of FM are seen, two prior to DMFP injection (0.3-0.5 mCi) and four after (0.4-1.4 mCi). d-amphetamine (0.4 mg/kg i.v.) was administered at 6850 s to elicit dopamine release. Cb = cerebellum, Ctx = cortex, Str = striatum, Th = thalamus.

6. Planned Experiments

6.1. *Schedule*

We expect to carry out this project on the following schedule: Sep 2005 – Feb 2006: methods development in rats, Mar 2006 – Aug 2006: methods development in rhesus, Sep 2006 – Feb 2007: neuromodulation study in rhesus, Mar 2007 – Aug 2007: simulations of human studies.

6.2. *Arterial Blood Sampling*

Arterial catheters will be placed by a surgical procedure[18]. Arterial blood will be sampled at 0.5 uL/s throughout and 1.3 uL/s for 2 min following injection of each [^{17}F]fluoromethane bolus, yielding 150 uL per 5 minute sample and total withdrawl of 3.5 mL. Blood will be analyzed on-line and in samples (Fig. 6).

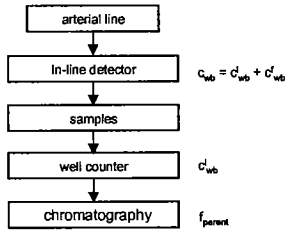

Figure 6. Separation of flow tracer and neuroligand input functions using arterial sampling. The in-line detector yields the whole blood TAC, $c_{wb}(t) = c^l_{wb}(t) + c^f_{wb}(t)$. Well counter measures of samples decayed by > 10 minutes yield the ligand whole blood TAC, $c^l_{wb}(t)$, so the flow tracer input function is given by $c^f_p(t) = c^f_{wb}(t) = c_{wb}(t) - c^l_{wb}(t)$. Chromatography measures of plasma give the unmetabolized parent fraction of neuroligand, $f_{parent}(t)$, and the input function is then $c^l_p(t) = f_{parent}(t) \times c^l_{wb}(t)$.

6.3. *Fitting Algorithm*

Based on the model presented in section 4.4, parameters will be determined by least squares fitting to observed data. The parameters of interest are Δk^f_1, *i.e.* the change in blood flow, and Δk^l_3, *i.e.* the change in DA receptor ligand binding. Statistical errors will be calculated by bootstrap and covariance between parameters will be determined.

6.4. *Amphetamine Challenge in Rhesus*

Five monkeys will each undergo 5 scanning sessions using the optimum protocol determined in pilot scans. In 3 sessions, the dual tracer method will be

used to correlate dopamine release and rCBF alteration in response to 0, 0.2, and 0.4 mg/kg amphetamine. In 2 additional sessions, 0.4 mg/kg amphetamine challenges will be delivered to each subject while imaging with [^{17}F]fluoromethane and [18F]DMFP alone. These sequential results will be compared to the simultaneously measured response to 0.4 mg/kg amphetamine.

7. Dreamed Experiments

If this dual tracer PET method proves feasible, it might be applied in a variety of studies. For example, it would be interesting to use other pharmacological challenges such as methylphenidate. Other modulatory neurotransmitters, *e.g.* serotonin, may be amenable to such techniques[19]. The ultimate goal of this development work is to use this technique in studies of behaving humans, for instance to probe the role of dopaminergic neuromodulation in attention.

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