ARTICLE
Positron Emission Tomography (PET) and Graphical Kinetic Data Analysis of the Dopamine Neurotransmitter System: An Exercise for an Undergraduate Laboratory Course

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Neuroimaging techniques, including positron emission tomography (PET), are widely used in clinical settings and in basic neuroscience research. Education in these methods and their applications may be incorporated into curricula to keep pace with this expanding field. Here, we have developed pedagogical materials on the fundamental principles of PET that incorporate a hands-on laboratory activity to view and analyze human brain scans. In this activity, students will use authentic PET brain scans generated from original research at Brookhaven National Laboratory (Volkow et al., 2009) to explore the neurobiological effects of a drug on the dopamine system.

We provide lecture and assignment materials (including a 50-minute PowerPoint presentation introducing PET concepts), written background information for students and instructors, and explicit instructions for a 4-hour, computer-based laboratory to interested educators. Also, we discuss our experience implementing this exercise as part of an advanced undergraduate laboratory course at Stony Brook University in 2010 and 2011.

Observing the living human brain is intriguing, and this laboratory is designed to illustrate how PET neuroimaging techniques are used to directly probe biological processes occurring in the living brain. Laboratory course modules on imaging techniques such as PET can pique the interest of students potentially interested in neuroscience careers, by exposing them to current research methods. This activity provides practical experience analyzing PET data using a graphical analysis method known as the Logan plot, and applies core neuropharmacology concepts. We hope that this manuscript inspires college instructors to incorporate education in PET neuroimaging into their courses.

Key words: teaching; positron emission tomography (PET); dopamine; raclopride; cocaine; radiotracer; addiction; dopamine transporter; modafinil; Logan plot; binding potential; distribution volume ratio (DVR); dissociation constant; laboratory course; human; imaging; education; college; pharmacology; neuropharmacology; neuroimaging; integrative; interactive learning

Positron Emission Tomography (PET) is used to locate and quantify radioactivity emitted by radiolabeled tracers throughout the living brain. Radiolabeled tracers are made by radioactively tagging compounds that bind to particular proteins expressed in the brain, such as dopamine receptors or transporters. In the experiments described here, PET is used to measure the effect of the wakefulness-promoting drug, modafinil (Provigil), on the brain's dopamine-based reward system. Many addictive substances excessively increase the brain dopamine levels; so a drug that substantially increases dopamine levels may also have the potential for abuse. Following administration of either modafinil or a placebo control, radiolabeled tracers that bind to dopamine receptors or transporters can be used to assay available binding sites for dopamine, and hence dopamine levels, in specific brain structures. Thus, radiolabeled tracers used in PET can provide a measurement of biological processes in the living brain.

In this exercise, students are given human PET scan data acquired under placebo or modafinil conditions, and they are asked to observe and quantify potential changes in dopamine levels. The PET data is registered to a structural template image of the human brain acquired with Magnetic Resonance Imaging (MRI), in order to facilitate the identification of brain Regions of Interest (ROIs). Using a free image analysis program (AMIDE; A Medical Imaging Data Examiner), students use the MRI template and human brain atlas to outline and identify dopaminergic brain regions (caudate, putamen, and nucleus accumbens), and then they measure the rise and fall of the radiotracer signal over time within these regions. Experimental variables include the use of PET scans from three different human subjects and the use of two different radiotracers, ^{11}C-raclopride or ^{11}C-cocaine, which bind to dopamine receptors and transporters, respectively. Further variation may arise from the students' selections or exact outlining of each brain region. Once the raw ROI data is obtained, a preformatted Microsoft Excel (2010) spreadsheet enables students to perform calculations and plot the data to reveal the effect of the drug treatment on dopamine levels via changes in radiotracer binding.

This lesson builds on and reinforces prior knowledge of brain structure and function, and introduces the physical principles underlying PET and the neuropharmacology of the dopamine system. In the laboratory course for which this module was developed, the exercise follows a unit on human neuroanatomy; however, students may consult human brain atlases (print or on-line) to aid in neuroanatomical identifications, if this unit is presented independently of lessons on neuroanatomy.

State-of-the-art neuroimaging techniques used in research can be challenging to incorporate into classroom settings. Certain factors, such as the complex equipment needed to run a PET scan and the potentially hazardous involvement of radioactive tracers, preclude a direct classroom experiment. However, our lesson circumvents these technical limitations by enabling students to examine and analyze authentic human PET data collected in a research setting. Although this exercise is executed on computers, our students were successfully engaged by the opportunity to work with data collected from living human subjects and the application of the methods to addiction research and its treatment.

Courses designed to teach neuroimaging methods to undergraduates are important, as these techniques become more widely used in science and medicine. Several educational manuscripts have already been published on functional MRI (fMRI) (Wilson, 2005; Hurd and Vincent, 2006). Here, we enable teachers to implement a PET neuroimaging laboratory exercise with minimal cost and set-up. All of the necessary materials are available upon request, including a preliminary lecture that covers the physical principles involved in PET, and the pharmacology of the drug and the radiotracers used. Our objective is to expand the available pedagogical materials in the imaging field by providing a feasible framework for instructors to educate undergraduates on PET neuroimaging.

STUDENT LEARNING OBJECTIVES
a) **Describe** the basic physics of PET
b) **Explain** pharmacological concepts about drug–target interactions and affinity using the different neuropharmacological properties of the two radiotracers examined, ^{11}C-raclopride and ^{11}C-cocaine
c) **Interpret** whether modafinil increases synaptic dopamine, and, if so, whether it also blocks dopamine transporters

MATERIALS AND METHODS
We incorporated this lesson on PET neuroimaging into a semester-long, 4-credit laboratory/lecture course in physiology and neurobiology (BIO335) at Stony Brook University. In this course, we began each week with a 50-minute lecture, which provided background information and outlined the goals for the laboratory experiment. For instructors, we provide PowerPoint slides and assessment questions (verbal and written) for a lecture, and a pre-laboratory introduction (Supplementary Material 1, available upon request). Instructor lecture notes for the PowerPoint presentations and answers to the assessment questions are provided in a supplementary document *Teacher Materials for Lecture and Laboratory* (Supplementary Material 2, available upon request).

- *Instructor's PowerPoint presentation*
  (Supplementary Material 1, available upon request)
- *Teacher Materials for Lecture and Laboratory*
  (Supplementary Material 2, available upon request)

In this 4-hour laboratory exercise, students work together in groups of two-to-three individuals. We had five laboratory sections with up to eighteen students in each section, and six groups per section. Our labs were taught with the help of graduate teaching assistants; however assistants are not required to execute this lesson.

PRE-LABORATORY PREPARATION
In preparation for the laboratory, students are required to read the *Neuroimaging Laboratory Manual*, specifically the section titled "PET Background Summary." It is also suggested that they read the primary research article on which this laboratory is based (Volkow et al., 2009); however, this is not mandatory. The *Neuroimaging Laboratory Manual* contains a vocabulary list, procedures for the laboratory exercise, and assignment questions. These materials are posted on an online distribution site at least one week before the lecture for students to access (our institution supports "Blackboard" http://www.blackboard.com/).

- *Neuroimaging Laboratory Manual* (Supplementary Material 3, available upon request)
- Primary research article:
  Volkow N, Fowler JS, Logan J, et al., (2009). Effects of Modafinil on Dopamine and Dopamine Transporters in the Male Human Brain: Clinical Implications, *JAMA*. 301(11):1148–1154

The PET data used in this exercise were first published in the *Journal of the American Medical Association* (Volkow et al., 2009), and were kindly shared by Drs. Joanna Fowler and Nora Volkow, who are pioneering research on human addiction with PET neuroimaging at Brookhaven National Laboratory (BNL) and at the National Institute for Drug Abuse (NIDA). The local Institutional Review Board (Committee on Research Involving Human Subjects, State University of New York at Stony Brook) approved the original study.

Six pairs of scans from three individual subjects are available from Volkow et al. (2009) for analysis (Supplementary Material 4, available upon request). The MRI template, MRI_template.nii, was originally downloaded from The Wellcome Trust Centre for Neuroimaging http://www.fil.ion.ucl.ac.uk/spm/, and it is used during the laboratory exercise to define brain regions associated with increased radiotracer binding.

- Three pairs of PET scans along with the MRI template image (Supplementary Material 4, approximatly 450 MB, available upon request), are loaded onto computers for students prior to the laboratory session:

  Group 1 – Subject 1 ^{11}C-raclopride placebo and modafinil
  Group 2 – Subject 1 ^{11}C-cocaine placebo and modafinil
  Group 3 – Subject 2 ^{11}C-raclopride placebo and modafinil
  Group 4 – Subject 2 ^{11}C-cocaine placebo and modafinil
  Group 5 – Subject 3 ^{11}C-raclopride placebo and modafinil
  Group 6 – Subject 3 ^{11}C-cocaine placebo and modafinil
  Groups 1 through 6 – MRI_template.nii

At least one laboratory computer with Microsoft Excel installed is required for each student group; students' personal computers can be substituted, if lab computers are not available. Access to a printer in the laboratory and memory sticks or internet access (for e-mailing) are also required to allow students to share their results and graphs with neighboring groups.

We structured the laboratory exercise so that the data viewing and analysis can be performed using AMIDE, a freely available image-viewing and analysis software program, and Microsoft Excel (2010). Instructions provided herein refer to the Windows version 1.0.0 of AMIDE; Mac and Linux versions of AMIDE are also available, though some instructions may have to be updated. The following software is required for the laboratory:

- AMIDE (A Medical Imaging Data Examiner, version 1.0.0), free image analysis software downloadable from http://amide.sourceforge.net/, should be installed on laboratory computers before the laboratory exercise starts. Download and installation takes less than 10 minutes.

AMIDE is used to observe the raw PET scans overlaid on top of the MRI scans, which shows the structural (MRI) and functional (PET) information simultaneously (Figure 1). AMIDE is also used to calculate radiotracer measurements in specific brain regions during the brain scan.
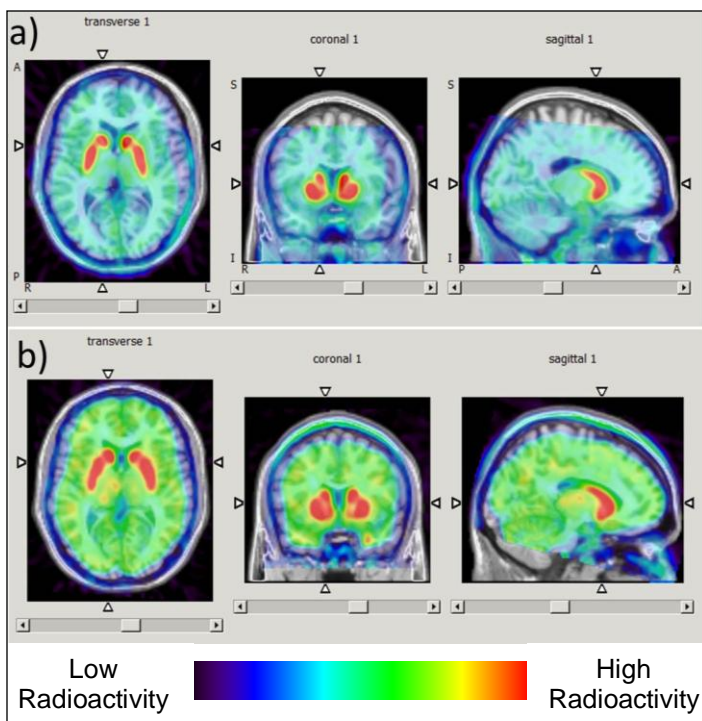

Low Radioactivity   High Radioactivity

Figure 1. Example of PET data overlaid on the MRI template, viewed in AMIDE software (left to right: transverse, coronal, and sagittal slices). *(a)* ^{11}C-Raclopride, placebo scans; *(b)* ^{11}C-cocaine, placebo scans. The color scale represents high radioactivity levels in red, located in the caudate, putamen, and nucleus accumbens, and lower levels in blue/green throughout the rest of the brain.

To aid in the qualitative and quantitative analysis of radioactivity levels, we have developed a preformatted *Excel Analysis Spreadsheet* for students to input and analyze their individual group data (Supplementary Material 5, available upon request). The spreadsheet contains separate tabs for analyzing ^{11}C-racopride and ^{11}C-cocaine data and an assignment for interpreting class data with statistical analyses. The preformatted spreadsheet can be loaded onto laboratory computers in advance.

- *Excel Analysis Spreadsheet* for students to input and analyze their individual group data (Supplementary Material 5, available upon request)

LABORATORY PROCEDURES
Students will work through the data analysis procedures described in the *Neuroimaging Laboratory Manual* in small groups (of 2–3 students). To encourage interactive learning and focused discussions of the physiological meaning of PET data, the *Neuroimaging Laboratory Manual* includes questions (boxed in red text) that the students can discuss in their small groups or with instructors. This can take place informally while students are working together, or the instructor can facilitate discussions.

The exercise is separated into two parts. Part I takes the students through visual examination of PET scans with semi-quantitative evaluation of the results. Part II employs fully quantitative, mathematical calculations in Microsoft Excel for measuring radiotracer binding in specific regions. We have made this distinction to provide a natural stopping point for instructors if a shorter version of this exercise is desired.

PART I: Qualitative observations of radioactivity over time in PET brain scans (Steps 1–5)

LAB OBJECTIVES Part I—Qualitative Observations
  a) **Identify** regions of the brain that express dopamine receptors (D_2/D_3 subtypes), and the dopamine active transporter (DAT), specifically the caudate, putamen, and nucleus accumbens, on the MRI template and the PET brain scans.
  b) **Measure** the effect of the drug modafinil on brain dopamine levels by calculating radioactivity levels in the caudate and putamen using AMIDE computer software.
  c) **Create** a graph the radiotracer activity over the time course of the scans ("Time Activity Curves" – TAC's) for qualitative interpretation by applying basic mathematical calculations in Microsoft Excel (using the *Excel Analysis Spreadsheet* provided) on raw data extracted from AMIDE.

STEP 1: Viewing the MRI Template in AMIDE
Students will begin by viewing the MRI template in AMIDE, and identifying key regions in the brain. They should be able to identify the caudate, putamen, nucleus accumbens, and cerebellum for this exercise. There are several atlases on-line for students to reference (see *Supplementary Material 2* "Helpful tips for lab exercise" for examples).

The caudate, putamen, and nucleus accumbens are rich in dopamine receptors and transporters. The cerebellum, in contrast, has little to no expression of dopamine receptors or transporters. Therefore, the cerebellum will be used in this exercise as a good measure of the background radioactivity level known as "non-specific radiotracer binding," as opposed to "specific binding," which occurs in target rich regions.

STEP 2: Drawing Regions of Interest
Next, students will use AMIDE to draw regions of interest (ROIs) around the caudate, putamen (in both the left and right hemispheres), and cerebellum on the MRI template. We excluded nucleus accumbens from the analysis here due to time constraints, but it could also be included at the discretion of the instructor.

STEP 3: Viewing the PET scan Images
Students are asked to view the PET data overlaid on top of the MRI scan, to show the structural (MRI) and functional (PET) information simultaneously (as shown in Figure 1). We spatially registered each subject's PET data to the MRI template to match them as closely as possible. However, individual brains vary, and the locations and dimensions for these structures may differ slightly between each of the subjects' scans.

In the data from the original study (Volkow et al., 2009), radioactivity values were continuously collected throughout the ~60-minute PET scan. The experimenter specifies time frames in which the radioactivity is averaged in 'bins,' in order to extract the most useful pharmacokinetic (or time-dependent) information about radiotracer movement throughout the brain. Scrolling through these time frames will allow students to see that levels of radioactivity in the brain rise and fall during the scan in specific regions.

STEP 4: Obtaining Mean ROI Data
After defining the ROIs, the students will use the program AMIDE to measure radioactivity values over the time course of the scan. AMIDE calculates the mean radioactivity and provides the numerical data in a spreadsheet format. The data is then cut-and-pasted from AMIDE into Microsoft Excel for analysis. To streamline the time it takes to do the analysis, we have developed an *Excel Analysis Spreadsheet* (Supplementary Materials 5) with separate tabs designated for ^{11}C-racopride and ^{11}C-cocaine data analysis. This spreadsheet facilitates the students' abilities to carry out the calculations (especially if Microsoft Excel is unfamiliar to them), and it also improves conceptualization of why each calculation is performed.
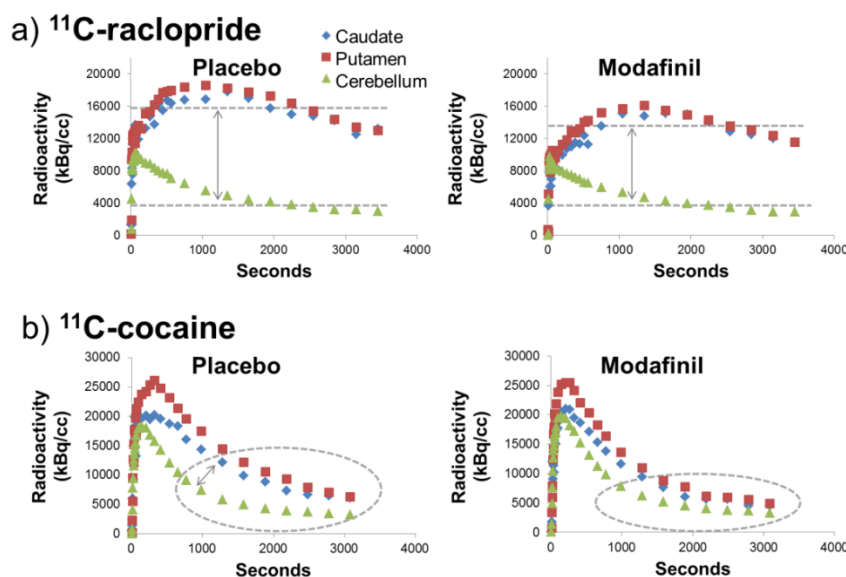
STEP 5: Graphing the Time Activity Curve
Students will create a graph that shows the changes in radioactivity during the scan that were observed when viewing the PET scan images in Step 3. They will plot the average ROI radioactivity data against time (midpoint of each frame), creating the Time Activity Curve (TAC), using Microsoft Excel and the *Excel Analysis Spreadsheet* provided. A different plot is generated for each condition, including placebo and modafinil with either ^{11}C-raclopride or ^{11}C-cocaine. Figure 2 shows an example plot for each condition.

DATA INTERPRETATION
The box in Figure 2, and subsequent text, contains key concepts to facilitate the interpretation of the TAC graphs.

(1) Blood flow transports radiotracers into the brain by diffusion through the blood-brain barrier into brain tissue. The shape of each TAC shows that radioactivity rises rapidly during the scan, reaching a peak. The rapid rise is generated by injecting the radiotracer directly into the blood

Figure 2. Time Activity Curves (TACs) for ^{11}C-raclopride (*a*) and ^{11}C-cocaine (*b*), placebo (left) and modafinil (right) scans. Box at right lists helpful pharmacology concepts for data interpretation. Graphs demonstrate that modafinil prevents some radiotracer binding compared to placebo (arrows in dashed areas). (Cerebellum, green triangles; caudate, blue diamonds; putamen, red squares).

Time Activity Curve Pharmacology:

1. Blood flow – carry radiotracers to brain

2. Passive diffusion – transportation of molecules into the brain tissue from blood vessels

3. Target specificity – radiotracer accumulates in tissue where target is present, or passively diffuses out of tissue (back into blood vessels)

4. Binding affinity – a higher affinity will result in stronger binding, and more accumulation

5. Drug effects – Modafinil can affect dopamine levels measured as a change in radioactivity compared to placebo

stream of the subject, where it is quickly carried by blood flow to the brain and body.

(2) Radiotracers <u>diffuse passively</u> between the blood stream and tissue. Within a few minutes of the scan, the radiotracer begins diffusing from the blood vessels into tissues, including the brain and the liver. The radiotracer is metabolized in the liver, thus slowly reducing its levels in the blood stream over time.

(3) Presence of <u>specific targets</u> (dopamine receptors or transporters) in a brain region allows the radiotracer to bind; lack of specific targets in a brain region results in passive diffusion of the radiotracer back into the blood stream. When the radiotracer diffuses into the cerebellum, there are no dopamine receptors or transporters for it to specifically bind to, so it diffuses into and out of the cerebellum brain tissue and blood vessels passively down a concentration gradient. However, when the radiotracers diffuse into the caudate, putamen, and nucleus accumbens, there are dopamine receptors and transporters present, so the radiotracers can bind to their targets rather than diffusing back into the blood stream.

(4) Radiotracers with strong <u>binding affinities</u> for their targets will tend to accumulate to higher levels than radiotracers with weaker binding affinities. In Figure 2, it is clear that the caudate and putamen ROIs look strikingly different between the ^{11}C-raclopride and ^{11}C-cocaine graphs (Figure 2, compare *a* and *b*). This is because ^{11}C-raclopride has a very low dissociation constant (K_d) for D_2/D_3 dopamine receptors, ~1.4 nM (Hall et al., 1988), whereas ^{11}C-cocaine has a relatively high K_d for dopamine transporters, ~16 - 210 nM (Volkow et al., 1995). The K_d is the inverse of the affinity, and thus a very low K_d indicates a very high affinity (or binding strength). Thus, ^{11}C-raclopride bound to a dopamine receptor will tend to remain bound, and thus more will accumulate over time, but ^{11}C-cocaine bound to a dopamine transporter may bind or dissociate dynamically, and will accumulate less over time.

(5) <u>Drugs</u> like modafinil can affect the synaptic concentration of dopamine, which is reflected as altered radiotracer binding compared to placebo.

In PET neuroimaging experiments, the radiotracers ^{11}C-raclopride and ^{11}C-cocaine are given in 'trace' amounts so as not to pharmacologically disrupt overall brain activity. The amount given is titrated so that the proportion of available protein targets, such as unoccupied dopamine receptors or transporters, can be revealed without significantly perturbing brain function.

Radiotracers can compete with endogenous ligands or exogenous drugs for binding to the same protein targets. For example, ^{11}C-raclopride is a dopamine receptor antagonist (for the D_2/D_3 subtype), and, thus, will compete with dopamine for binding to dopamine receptors. If there is a high concentration of endogenous dopamine present in the brain, then there will be fewer opportunities for ^{11}C-raclopride molecules to bind dopamine receptors, since many receptors will already be occupied by dopamine. Thus, a reduction in ^{11}C-raclopride binding indicates increased dopamine levels in the synapses.

The dashed areas in Figure 2*a* visually delineate the differences in radiotracer quantities between the placebo and modafinil conditions for ^{11}C-raclopride scans. Focusing on later time points in the graph, where equilibrium is reached, there is a larger difference between the mean radioactivity values in the cerebellum and caudate/putamen in the placebo condition compared to modafinil, suggesting that the concentration of synaptic dopamine is enhanced when modafinil is present.

In the same way, some drugs can bind to transporters and affect their functioning. For example, cocaine can bind to the dopamine transporter and block the ability of the transporters to recycle dopamine. High concentrations of cocaine cause an accumulation of dopamine in the synapse and facilitate dopamine neurotransmission. However, as a radiotracer, ^{11}C-cocaine reveals the number of dopamine transporters with unoccupied binding sites that are available for radiotracer binding, with limited overall impact on dopamine neurotransmission.

The dashed ellipsoids in Figure 2*b* visually delineate differences for the ^{11}C-cocaine scans in the presence of modafinil. Some ^{11}C-cocaine radiotracer binding is prevented by modafinil, suggesting an interaction between modafinil and the dopamine transporter.

The overall qualitative observations shown in Figure 2 suggest that modafinil blocks the dopamine transporter and, consequently, synaptic dopamine increases. Importantly, data from other experiments coincide with these findings, indicating that modafinil does block the dopamine transporter (Zolkowska et al., 2009; Schmitt and Reith, 2011). While the TACs can reveal qualitative changes between placebo and modafinil, a further graphical analysis— – the Logan plot— – is needed to quantify changes, and to compare results between subjects.
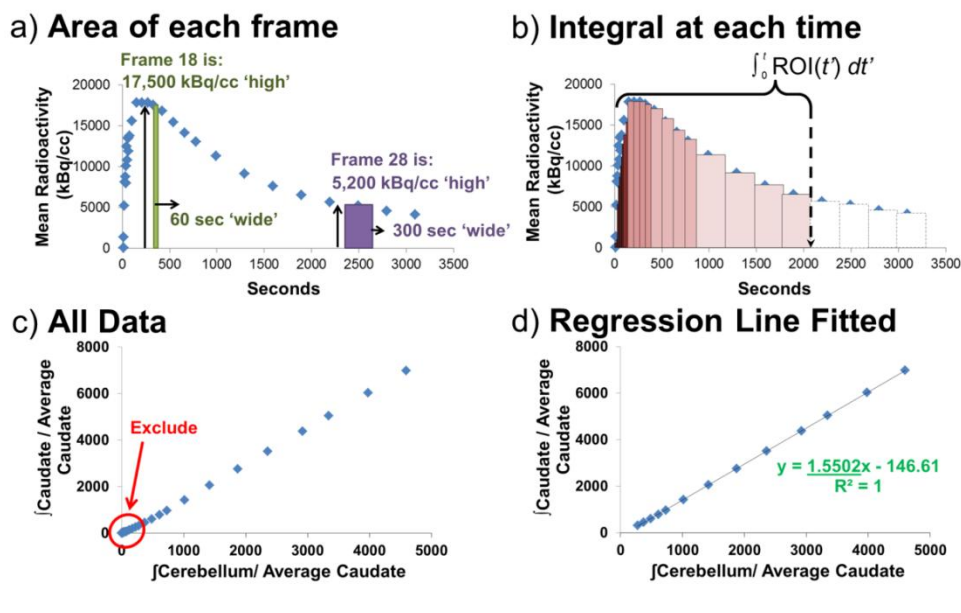
PART II: Quantification of radioactivity levels in the brain over time in PET scans (Steps 6–9)

LAB OBJECTIVES Part II—Quantitative Measurements
   a) **Compute** the Distribution Volume Ratio (DVR) and Binding Potential (BP_{ND}), which provides a quantitative measure of radiotracer binding, by executing a graphical linear regression analysis (Logan plot) with the *Excel Analysis Spreadsheet*.
   b) **Compare** the DVR and BP_{ND} for each experimental condition (placebo/modafinil; caudate/putamen; ^{11}C-cocaine/^{11}C-raclopride).
   c) **Evaluate** the class data using a paired Student's *t*-test to determine whether statistically significant differences exist between placebo and modafinil conditions for each region, and radiotracer.

STEP 6: Area under the Curve
In Part I of this exercise, students created TAC's by measuring the mean radioactivity concentrations present in each ROI (caudate and putamen) and a reference region (REF; cerebellum) in discrete time frames during the scan. Students will use these data to approximate the integral (or

Figure 3. Logan plot analysis to obtain the Distribution Volume Ratio (DVR). (*a,b*) Calculating areas under time activity curves. (*a*) Each time frame has an area calculated by multiplying the width (in seconds) and height (mean radioactivity in kBq/cc). (*b*) The integral (sum) at each time point is calculated by adding the areas of each rectangle from 0 to *t*. An example is shown for *t* = 2,040 seconds. The integral is estimated by adding up the shaded rectangles (each shade represents different time frame durations). (*c,d*) Example of a Logan plot, all *x* and *y* data are shown in panel *c*. Notice the data is linear, except for some points near the origin (circled in red). (*d*) Regression line is added, and early points are excluded. Equation of the line is given in green, with the slope of the line underlined. Example is from ^{11}C-cocaine data; note that ^{11}C-raclopride data shows more dramatic non-linearity in early points. Box at right lists key ideas on Logan plot calculations.

area-under-the-curve) for each TAC by summing the individual rectangles making up each time frame. The length of each frame is measured in time and the height is measured as mean radioactivity concentration. Figure 3*a,b* visually demonstrates the summation process to estimate the integral at each time for an example TAC.

Students will use the estimated integrals as independent variables in equation 1, known as the Logan Plot. The Logan plot is one commonly used PET data analysis method that can reveal quantitative changes between placebo and experimental conditions, and is a means to accurately compare results between subjects (Logan et al., 1990, 1996; Logan, 2000). Students will follow the step-by-step calculations in the *Excel Analysis Spreadsheet* to determine each mathematical variable in Logan's equation [1], beginning with calculating the area-under-the-curves.

$$\frac{\int_0^t ROI(t')dt'}{ROI(t)} = DVR \ \frac{\int_0^t REF(t')dt'}{ROI(t)} + int' \qquad [1]$$

STEP 7: Graphical Kinetic Model—the Logan Plot
Students will notice that Logan's equation [1] is a linear equation taking the form, $y = m\,x + b$. The instructor can circle the multi-component *x* and *y* terms to facilitate recognition. The *m* (or slope) of Logan's equation is defined as the Distribution Volume Ratio (DVR).

The DVR represents the ratio of specific to non-specific binding of the radiotracer in tissue, and is therefore a

quantitative measure of the number of targets available for the radiotracer to bind.

Students will compute the DVR in several steps. First, students will plug in the caudate (or putamen) integrated values into equation 1 as "$\int ROI$", and the integrated values for the cerebellum as the reference region ("$\int REF$"). Logan uses a reference region in the equation to account for the non-specific binding that occurs in tissue regardless of the presence of radiotracer target molecules. Thus normalizing to the cerebellum, provides a good estimate of true, specific radiotracer binding in the caudate or putamen at dopamine receptors or transporters. Secondly, the terms $\int ROI$ and $\int REF$ are divided into the caudate (or putamen) mean radioactivity values at each time point yielding a list of values for y and x, respectively. Thirdly, students will plot the list of x and y values using Excel. Finally, they will perform a regression analysis to determine the equation of best fit line.

As described above, the slope is equal to the DVR. Students should exclude time points early in the graph from the regression analysis, as shown in Figure 3*c*, but include later points. The reason for focusing the regression analysis on the later points is because radiotracer binding has most closely reached equilibrium and therefore provides the best approximation for how many sites are available for binding. In Figure 3*d*, the DVR equals 1.5502 (unit-less).

STEP 8: Binding Potential
Students will compute the binding potential (BP$_{ND}$), another

term used to describe how much radiotracer can 'potentially' bind to its target, (Innis et al., 2007). Binding potential is traditionally determined experimentally using *in vitro* binding assays by measuring the number of radiotracer molecules bound to the target divided by the free unbound radiotracer at equilibrium. BP_{ND} for *in vivo* neuroimaging studies is related to the DVR by equation [2], which accounts for the not-specifically bound or "non-displaceable" ($_{ND}$) fraction.

$$BP_{ND} = DVR - 1 \qquad [2]$$

Thus, the DVR calculated in Figure 3*d* (1.5502) yields a BP_{ND} of 0.5502. The pre-laboratory PowerPoint presentation explains the key concepts relating the DVR to BP_{ND}. Derivation of the relationship between the DVR and BP_{ND} is described in the *Neuroimaging Laboratory Manual*, and a further explanation is provided in the "Conceptual Pitfalls" section of *Teacher Materials for Lecture and Laboratory*.

A change in binding potential between the placebo and modafinil scans can be calculated using equation [3].

$$\% \ Change \ BP_{ND} = \frac{Experimental - Baseline}{Baseline} \ x \ 100\% \qquad [3]$$

Table 1 shows sample results for the percent change in binding potential (BP_{ND}).

	^{11}C-Raclopride	^{11}C-Cocaine
Placebo	2.0975	0.8348
Modafinil	1.9093	0.4743
%Change BP_{ND}	-8.97	-43.18

Table 1. Examples of BP_{ND} results for putamen from Subject 2.

STEP 9: Statistical Analysis of Class data
Students will analyze the class data for percent change in BP_{ND} and evaluate potential statistical differences between placebo and modafinil conditions, among the three subjects investigated by the students. The third tabbed section in the *Excel Analysis Spreadsheet* facilitates calculations with a Paired Student's t-test. Also, average percent change in BP_{ND} is calculated for each experimental condition, and compared to the published values (Volkow et al., 2009). The average percent change for ^{11}C-cocaine in Volkow et al. (2009) is 53.8% in the caudate, (43.9%–63.6%; $p<0.001$) and 47.2% in the putamen (39.1%–55.4%; $p<0.001$). The average percent change for ^{11}C-raclopride is 6.1% in the caudate (1.5%–10.8%; $p=0.02$) and 6.7% in the putamen (3.2%–10.3%; $p=0.002$).

The class results will vary and may not exactly match the published data, depending on several factors. For example, less accurate ROI shapes and placement on the PET images can reduce the accuracy of the measured BP_{ND}, particularly if the ROI's contain regions adjacent to the caudate and putamen. Students may or may not have reached a significant $p<0.05$ value with the class data, but it should be noted that they have only analyzed data for

three subjects, whereas the original paper was based on data from 10 subjects. Analyzing additional subjects would give the *t*-test more statistical power, and thus would more closely match the published data.

DATA INTERPRETATION
The expected result for ^{11}C-raclopride binding is that the BP_{ND} should decrease when modafinil is given to the subject compared to the placebo condition. Similarly, the expected result for ^{11}C-cocaine binding is that the BP_{ND} should also decrease when modafinil is given.

Decreases in ^{11}C-raclopride binding reflect increases in extracellular, endogenous dopamine. Compared to placebo, the data suggests that modafinil caused an increase in synaptic dopamine release. Therefore, as more endogenous dopamine was present in the synapse and extracellular space, there was more competition for any available dopamine receptors in the synapse. Thus, a measured reduction in ^{11}C-raclopride binding in the modafinil scan compared to placebo would suggest that modafinil caused an increase in dopamine.

Decreases in ^{11}C-cocaine binding provide evidence for a potential mechanism of how modafinil can increase synaptic dopamine. Since ^{11}C-cocaine binding is reduced in the presence of modafinil, this suggests that modafinil can interact with the DAT. Presumably, modafinil binds to the DAT and blocks its function, thereby preventing recycling of dopamine out of the synapse. Therefore, the ^{11}C-cocaine data suggests that modafinil can also bind to the DAT and potentially prevent dopamine recycling, thereby allowing more dopamine to remain in the synapse potentiating dopaminergic synaptic transmission.

ASSESSMENT
Listed below are the modes of assessment that we used for this Neuroimaging laboratory exercise and as part of the students overall course grade for BIO335.

Grade for Neuroimaging laboratory exercise (out of 100%):
- Laboratory Manual questions (50%)
- Graphs (four TAC's, two Logan Plots) (24%)
- Completed Class Results & Statistics (5%)
- Data Analysis questions (21%)

Part of overall course participation grade:
- Lecture clicker questions
- Pre-laboratory written quiz
- Verbal questions during laboratory (non-graded)

In the lecture, we incorporated the classroom response system (also known as clickers) from Turning Technologies (http://www.turningtechnologies.com). With clickers, instructors can ask students questions during lectures (verbally, or in PowerPoint presentations), poll their responses, calculate the percentage of students responding correctly in real time during the lecture, and also apply the results toward the student's overall grade. In our class, students were required to bring their clickers to lecture in order to receive 2 points for correct answers, 1 point for incorrect answers, or 0 points for no answer (or

absent student). We provide example clicker questions that can be incorporated into the lecture.

For the laboratory exercise, we have several avenues of formal and informal assessment. First, we administer a short quiz prior to starting the laboratory procedures, to assess the students' preparation in reading background materials and understanding key concepts from the lecture. Second, we use informal verbal assessment by walking around the laboratory going to each group discussing their progress and fielding questions. We also use informal visual assessment by asking each group to point out key brain regions involved in the reward system (caudate, putamen, and nucleus accumbens) on the MRI and PET scans. Third, we ask students to complete the *Excel Analysis Spreadsheet* with their own results, and turn in six graphs including the Time Activity Curves (TACs) and the Logan plots. Fourth, we ask students to compile the class results and run statistical analyses. And, fifth, we require students to independently complete the questions provided at the end of the *Neuroimaging Laboratory Manual*, which consists of the 10 boxed questions (in red text) that students can discuss together and then independently write their responses, along with additional questions related to data analysis interpretation and conclusions.

For educators interested in implementing this module, we have prepared *Teacher Materials for Lecture and Laboratory,* Supplementary Material 2, available upon request. These materials include a grading rubric, answers and explanations to the clicker questions from lecture, example graphs and tables, and descriptive answers to the pre-laboratory quiz and written questions.

RESULTS AND DISCUSSION

Upon successful completion of this lesson, students are expected to achieve the three learning objectives described in the introduction. Based on our experience executing this course, we provide a discussion of how successful this exercise was in achieving these goals.

STUDENT EVALUATION OF COURSE
We developed and implemented this laboratory module as part of an undergraduate course at Stony Brook University, BIO335 Animal and Neurophysiology Laboratory, which was taught in 2010 and 2011. There were a total of 180 students from the two semesters participating in the course, and these students provided feedback through student evaluations, which are completed at the end of the semester.

The overall feedback on the subject matter was very positive. Students were asked to rate on a scale of 1 (strongly agree) to 7 (strongly disagree) whether they agreed or disagreed with particular statements. When students were asked if the subject matter was clear and understandable, whether the instructor made the objectives and procedures of laboratory clear, and whether the work was challenging and worthwhile, on average 50% of students strongly agreed (score of 1), 34% mostly agreed (score of 2), 9% agreed (score of 3), 5% were neutral (score of 4), and less than 2% disagreed or strongly disagreed (score's 5–7).

We also received written feedback from the students for the lecture and laboratory. For the lecture, students described the topic using the following phrases; "informative and interesting," "cool," "[presentation was] explained very well and was interesting to listen to, especially since I am interested in the topic." One student noted that we "did a good job of explaining the physics side of the lab without going too much into it," and several students commented that we "did a great job of going over all the necessary background info for lab." There were both positive and negative responses toward the clicker questions, as several students noted "the clicker questions were very helpful" but others suggested that we should "ask fewer clicker questions." For the laboratory, students described that "learning about techniques and the pharmacology behind neuroimaging was cool," and that we presented an "interesting lab relevant to medicine and current clinical imaging." Also, students noted that "the Microsoft Excel spreadsheet [*Excel Analysis Spreadsheet*] was very easy to work with and the instructions were very clear," and one student wrote "the lab was fun and very informative, I feel like I learned a lot about how imaging studies are done and the concepts behind them."

Some criticisms written by the students suggested that a lack of hands-on experiments was a drawback. Comments suggested that that the laboratory "wasn't very engaging as there were no hands-on experiments;" "some trouble and time was spent figuring out computer stuff than actual science;" "the lab was overly complex and put too much emphasis on using the program in Excel rather than having us understand the underlying physiology;" and "[we were] just entering numbers into a computer and not truly learning much about physiology." Several students in the 2010 class noted a deficiency in Excel; therefore for the 2011 class we included more detail in the *Neuroimaging Laboratory Manual* for using Excel at each step, and we included the *Excel Analysis Spreadsheet* to facilitate organization of data. These additions significantly facilitated students' completion of the analysis and reduced the frequency of criticisms describing difficulty using Excel in 2011. In order to improve "the focus on the biology and not the computer science aspects of PET analysis," as another student put it, we encouraged student discussion of each key pharmacological concept during the *Neuroimaging Laboratory Manual* procedures. Also, to measure whether students would be able to generalize their interpretations, we asked them to compare their results with a neighboring group using a different radiotracer and challenged them with the Data Analysis Questions (in the *Neuroimaging Laboratory Manual* assignment). This comparison exercise facilitated their overall interpretation of the data and ability to draw conclusions and make generalizations about radioligands used in a PET experiment.

We undertook a considerable effort to make this exercise as interactive and exciting as possible for students learning about neuroimaging, and found that the majority (93%) of students agreed that we were successful in meeting this goal. Unfortunately, it is not possible to run an actual PET experiment in the laboratory, as this would

involve expensive equipment and exposure to radioactivity. However, one potential way to improve the laboratory in the future would be to create a virtual PET experiment using a computer simulation that the students could view and interact with, in addition to conducting the PET data analysis. Simulations have been successfully developed at Rice University for example, where a computer game and virtual experiment was created for youth to learn about the effects of drugs on physiology (Miller et al., 2006; Klisch et al., 2012).

TEACHER EVALUATION OF STUDENT LEARNING
Grades on the laboratory results and homework assignments were used to assess if we were successful in educating students on PET neuroimaging. Overall, we were satisfied that students completed the basic goals, which included identification of dopaminergic regions in the human brain and measuring the radioactivity in these regions using the Logan plot. Many students were able to master interpretation of PET results, including how ^{11}C-cocaine and ^{11}C-raclopride can be used to measure the effect of modafinil on the brain, and what factors contribute to modafinil's mechanism of action. Student success was demonstrated by average grades, which were 85.1% ± 14.5 in 2010 and 84.3% ± 10.8 in 2011. These grades were consistent and slightly higher than the overall average scores for the class (averaging all 12 weeks' worth of laboratory assignments), which were 81.2% ± 13.2 in 2010 and 79.9% ± 8.0 in 2011. Our results demonstrated that the students successfully learned the topics outlined in this manuscript.

SUMMARY
Neuroimaging has become an integral part of clinical diagnosis and basic neuroscience research. The exercise described here provides a hands-on, computer-based platform for instructing students on Positron Emission Tomography (PET) neuroimaging and pharmacology concepts related to dopamine neurotransmission in the human brain.

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