

FOCUS: MICROSCOPY AND IMAGING

How to Design PET Experiments to Study Neurochemistry: Application to Alcoholism

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Positron Emission Tomography (PET†) (and the related Single Photon Emission Computed Tomography) is a powerful imaging tool with a molecular specificity and sensitivity that are unique among imaging modalities. PET excels in the study of neurochemistry in three ways: 1) It can detect and quantify neuroreceptor molecules; 2) it can detect and quantify changes in neurotransmitters; and 3) it can detect and quantify exogenous drugs delivered to the brain. To carry out any of these applications, the user must harness the power of kinetic modeling. Further, the quality of the information gained is only as good as the soundness of the experimental design. This article reviews the concepts behind the three main uses of PET, the rationale behind kinetic modeling of PET data, and some of the key considerations when planning a PET experiment. Finally, some examples of PET imaging related to the study of alcoholism are discussed and critiqued.

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†Abbreviations: PET, positron emission tomography; SPECT, single photon computed tomography; NMSP, N-methylspiperone; NTX, naltrexone; BP, binding potential; CS, conditioned stimulus; B/I, bolus plus infusion; PD, Parkinson's disease; DA, dopamine; D2, dopamine receptor type 2; MOR, DOR, KOR, mu, delta, kappa opioid receptor; TAC, time-activity curve; SRTM, simplified reference tissue model.

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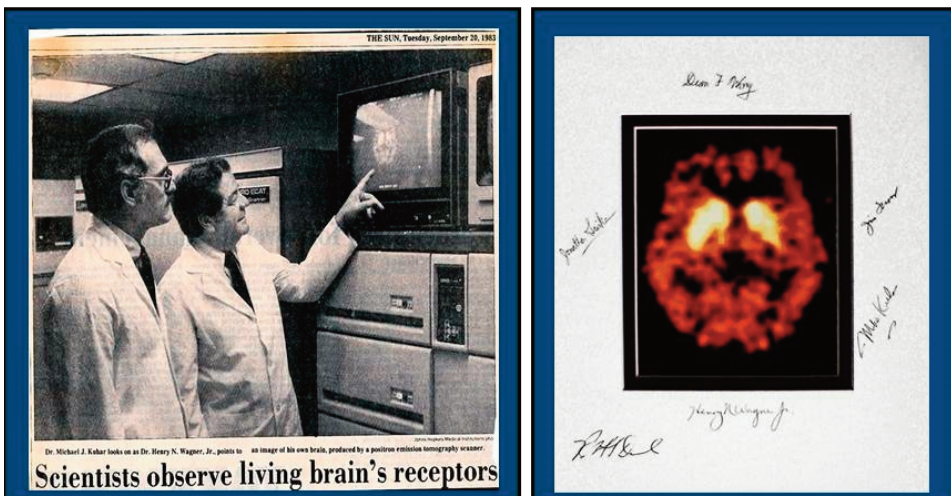


Figure 1. (Left) Newspaper clipping from the Baltimore Sun, Sept 20, 1983, shows senior authors Drs. Henry Wagner, Jr. and Mike Kuhar observing the first images of D2 receptors in a human brain, *in vivo*. (Right) A keepsake from the experiment adorns the offices of many of the landmark study's participants. Signatures, from the center bottom going clockwise, Wagner, Robert Dannals, Joanthan Links, Dean F. Wong, Jim Frost, and Kuhar. Photos courtesy of M. Kuhar.

INTRODUCTION

Historical Background

The era of imaging neuroreceptors in humans with positron emission tomography (PET) was ushered in by Wagner et al. (1983) with a report in *Science* showing the first human brain scan of dopamine receptors [1]. The tracer was N-methylspiperone (NMSP) tagged with carbon-11. The brain that was scanned belonged to one of the authors. Ethical concerns notwithstanding, this act placed the researchers in the good company of famous scientists throughout history who had experimented on themselves. The publication of this paper excited the field and garnered some publicity as well (Figure 1). Although the study did not employ the quantitative analysis techniques we describe below, it presaged some of the key concepts. Namely: 1) early images contain mostly blood flow information; 2) late images primarily reflect binding; 3) radioactive tracer in the target tissue can be “free” or “bound,” which often necessitates the examination of a “reference region,” which is devoid of receptor sites; and 4) co-injection of radiolabelled tracer with an *excess* of unlabeled

tracer can be used to prevent radiotracer from binding and thus measure unbound (aka, non-displaceable) signal by itself. Injection of excess unlabeled tracer is generally not performed in humans; in this case, it was done in baboons. As we discuss below, the ability to use PET to measure receptor number or some index thereof opens up additional measurement possibilities that take advantage of a key concept: competition. In the Wagner paper, the competition was between hot (labeled) and cold (unlabeled) tracer [1]. In another ground-breaking paper that followed it, the competition was between a radiotracer and an unlabeled neuroleptic drug [2]. Farde and colleagues did what amounts to the first drug occupancy study with PET using the D₂/D₃ specific antagonist tracer, [¹¹C]raclopride, in 1986. Their paper was intended to examine the occupancy level of drugs for schizophrenia in treated schizophrenics by examining the degree of tracer blocking at the dopamine D2 receptor sites achieved by each patient's respective drug. Whereas Wagner et al. could examine the difference between a baboon at baseline and following a co-injection of tracer with an excess of cold NMSP [1], Farde et al. did not ask their patients to go

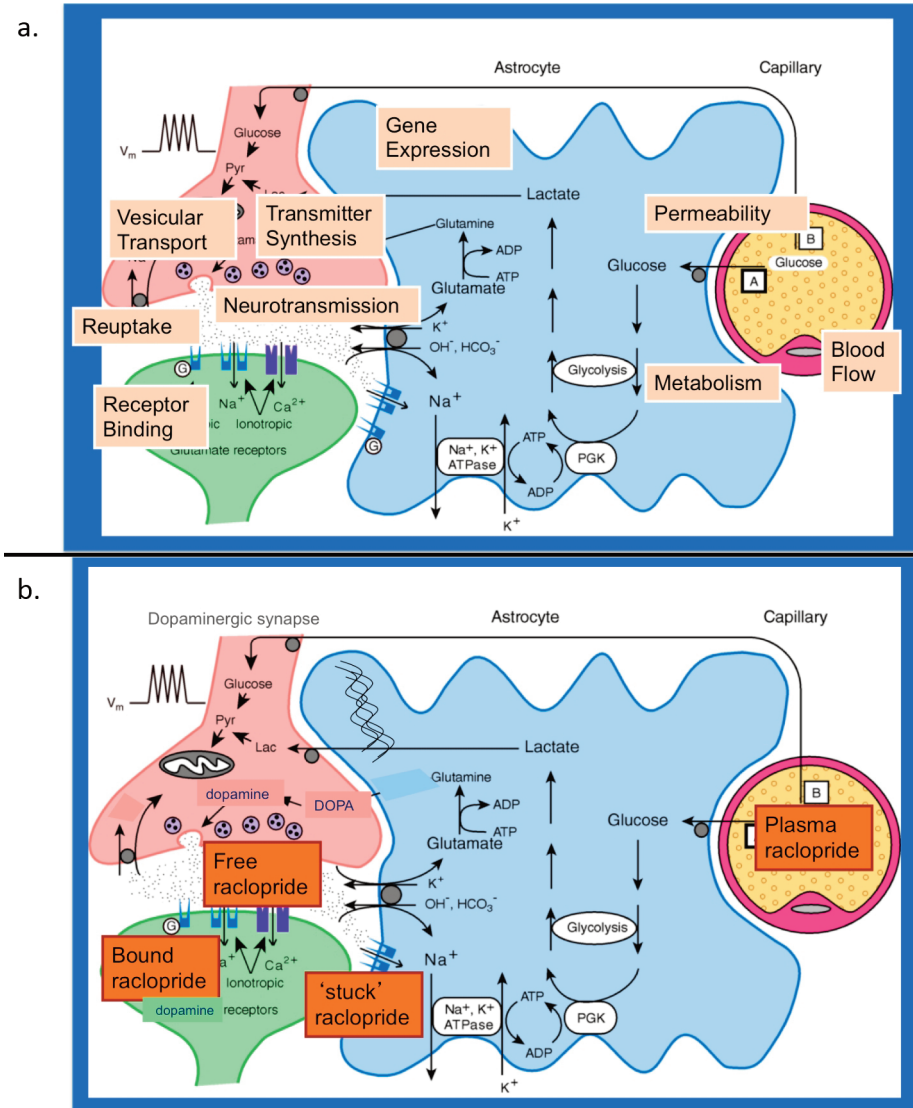


Figure 2. a) Molecular processes that can be imaged with the appropriate PET ligand. Figure modified [61]. **b)** Possible states of an injected radiotracer, [¹¹C]raclopride and DA D2 receptors used as an example. The states can be thought of as distinct, interconnected pools. Figure modified [61].

off medication to get a baseline measurement of tracer binding [2]. So how did they make an assessment of drug occupancy, which requires at least two measurements? They extrapolated what baseline binding might have been in their schizophrenics from a cohort of control subjects. Provocatively, they found that three schizophrenics undergoing (successful) treatment with different drugs all had receptor occupancies of very similar levels. Their approach would

likely not pass muster today, but at the time, the paper was highly innovative, and it foreshadowed one of the major usages of PET and neuroreceptor tracers: measuring target occupancy by drugs in people.

Basics

Molecular Specificity

PET is unique among medical imaging modalities for its exquisite molecular specificity. From this specificity, PET derives its

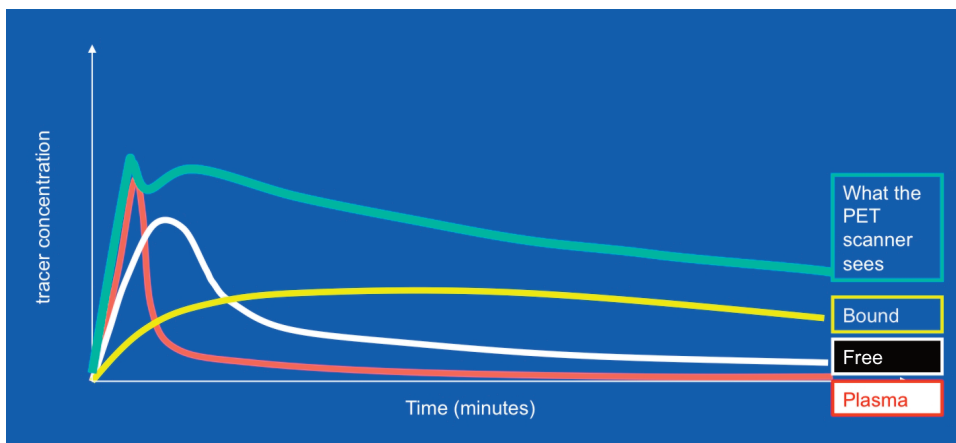


Figure 3. Different pools (compartments) of tracer activity are distinguishable by their different kinetics. Plasma activity (red) is cleared fastest. The free tracer pool (white) is slightly slower. The bound tracer pool (yellow) persists for longest. The PET scanner measures the sum of all the radiactivity (green).

unique ability to image highly selective biological processes — that is, to act as a *functional* imaging modality. In the brain (and everywhere in the body), different processes are facilitated by highly specialized molecules. Individual enzyme molecules exist to catalyze highly selective and uni-purpose biochemical reactions. Unique receptors and transporters exist to bind highly specialized endogenous ligands and carry out unique physiological functions. Some of the functions of interest that are controlled by individual molecules and which we may want to image are shown in Figure 2a. PET can image any of these molecular targets provided two obstacles have been overcome. First, a tracer molecule that binds or interacts with the target site must exist and be labeled with a positron emitting isotope (typically, carbon-11 or fluorine-18). Second, it must be possible to deliver the tracer to the target site. In brain imaging, the most likely cause of tracer failure is the inability of the tracer to cross the blood brain barrier to access the target.

Many Tracers for Many Targets

At this writing, there are tracers for many of the common neurotransmitter receptor sites: dopamine (D_2/D_3 and D_1), serotonin ($5HT_{1a}$, $5HT_{1b}$, $5HT_4$), and transporter sites (DAT, SERT, NET). Tracers generally arise through one of three pathways: 1) ra-

diolabeling of a dye or other molecule that is known to be selective for a particular target of interest (e.g., $[^{11}C]PIB$, a tracer for beta-amyloid, arose from the radiolabeling of thioflavin-T) [3]; 2) radiolabeling of a candidate drug for the target molecule of interest (e.g., $[^{11}C]$ erlotinib was synthesized as a tracer for the kinase domain of the epidermal growth factor receptor — commonly expressed in non-small cell lung cancer [4,5]). Candidate compounds may also have been failed drugs (adverse drug side-effects on patients, kinetics too rapid to sustain clinically useful levels in blood and tissue) but make good tracers (no adverse side effects, because tracers are given in micro-dose amounts, favorably rapid kinetics); and 3) de novo design of new PET tracer based on knowledge of the structure of the target molecule.

Specific Binding vs. Nonspecific Background

Tracers are administered to subjects intravenously and travel to the brain via the circulation. Once they traverse the blood brain barrier (typically by passive diffusion), they can follow three possible fates. Some tracer molecules remain free (unbound), eventually clear back to the vasculature, and are removed from the organ. Other tracer molecules, once inside the tissue, may bind to the specific target of interest. Because no tracers

are perfectly ideal in their behavior, some molecules are bound nonspecifically (nondisplaceably) before clearing from the tissue. Thus, in toto, radioactive emissions that are detected by the PET scanner are a (time-varying) sum of emissions of radio-isotopes on tracer molecules (e.g., [^{11}C]raclopride) in all four different possible states: blood-borne, free in tissue, specifically bound to a receptor or other target molecule (e.g., dopamine D_2/D_3 receptors), or nonspecifically bound (Figure 2b). The PET scanner records all of these emissions indiscriminately. Nothing about the photons that are emitted from an annihilation event in the blood or tissue makes their original state knowable from the detected signal. Thus, on any given *static* PET image (a single image summed over a time frame), the desired signal — i.e., the amount of specifically bound tracer — cannot be discerned easily because the signal is confounded by background activity coming from tracer in its three other possible states.

The one thing that allows us to differentiate the binding from the background is the difference in temporal behavior of the various tracer states. The persistence of activity (in a sense, the residence time) in each of the plasma, free, bound, and nonspecific pools is different (see curves in Figure 3). Thus, to identify the specific binding component of the total PET signal (green curve on Figure 3, also called a time-activity curve (TAC)), we must a) acquire dynamic data (over many time frames); b) identify an input function to the system (either plasma radioactivity or image-derived); and c) apply a mathematical model to separate the dynamic data into its constituent parts.

Binding Potential as Endpoint

The most common endpoint for imaging neuroreceptor or neurotransmitter targets with PET is the compound parameter: binding potential (BP). The term was first introduced by Mintun and is equivalent to the steady state ratio in the target tissue of specifically bound tracer to free tracer [6]. Binding potential is a “compound” parameter, because it is equivalent to the ratio of individual rate constants (specifically, the

association and dissociation rate constants). The rate constants arise in the standard compartmental model used to describe a TAC measured in a region of interest in the dynamic PET images. Readers should be aware that there are a few variations on the definition of binding potential [7]. The definitions differ by what data are used as the input function to drive the particular kinetic model and by what assumptions are made. Nevertheless, the general principle can be stated: BP can be estimated as the steady state ratio of bound to free tracer. BP is also proportional to the available binding sites and inversely proportional to the equilibrium dissociation constant, K_D , of the tracer for the binding site. The former concept is diagrammed in Figure 4a. We see that there are four species of interest in imaging neuroreceptor targets: First, the receptor; second, the tracer molecule that binds to the target and emits a positron; third, the unlabeled tracer which also binds to the target but emits no positron; and fourth, the endogenous ligand that is also specific for the target but (naturally) emits no positron.

Changes in Receptor Number

As previously stated, BP is proportional to number of available receptor binding sites (B_{max}) and typically serves as a convenient surrogate for receptor density, provided the proportionality constant can be taken as a constant across the groups or conditions being compared. When BP is estimated from dynamic data using the arterial plasma concentration of tracer as the input function, the proportionality between BP and B_{max} is simply: $1/K_D$ (i.e., $\text{BP} = B_{\text{max}}/K_D$), where BP is the binding potential between the tracer and target, B_{max} is the number of available receptor binding sites, and K_D is the equilibrium dissociation constant of the tracer for the binding site. Perhaps the most common use of BP as an endpoint is to assay receptor density (e.g., dopamine D_2R) in two groups of subjects (e.g., healthy controls and cocaine addicts) and compare them [8,9]. In such a case, the density of receptors may be believed to have a direct functional role in a disease process. Alternatively, receptor number can be a surrogate marker for a number of

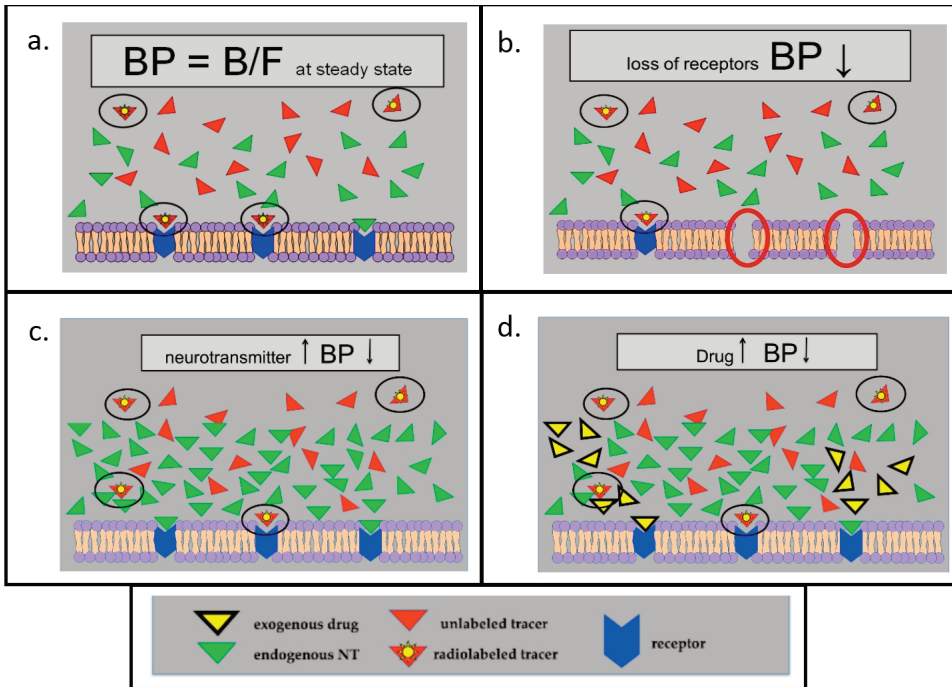


Figure 4. **a)** Binding potential depicted as bound over free tracer (red with star) at steady state. Receptor (or transporter) molecules (blue) may be embedded in a cell membrane. Two other species compete with tracer for limited binding sites: cold tracer (red), endogenous ligand (green). **b)** Lower binding potential reflects lower receptor density than in 4a. **c)** Elevation of endogenous neurotransmitter (green triangles) blocks available receptors and is detected as a reduction in BP. **d)** Effect of exogenous drug on binding potential. Drug (yellow triangles) occupies some receptor sites reducing available binding sites and then reducing BP.

functioning neurons. Consider Parkinson's disease (PD), which involves loss of nigro-striatal connections. Because functioning nigro-striatal projections contain D2 receptors and dopamine transporters on their striatal terminals, absence of such sites in a PET scan is indicative of disease progression and attendant loss of neurons. Low dopamine receptors and low dopamine transporters have each been demonstrated with either [^{11}C]raclopride or [^{11}C]CFT, respectively, by comparing the BP for healthy controls to that of PD patients [10,11]. The schematic in Figure 4b represents the case of low BP caused by low receptors (Figure 4b should not be interpreted too literally; e.g., in the case of PD, the entire cell membrane along with the receptors might be missing).

Changes in Endogenous Neurotransmitter

Another popular use of BP is as a measure of steady state neurotransmitter level.

Such measurements are typically made by comparing BP in the same subject at baseline and in a drug or treatment condition. This can be done via two paired bolus injections of tracer or via one bolus plus infusion of tracer. Typically, a drug will be given prior to the PET scan. The drug (e.g., cocaine, methylphenidate, amphetamine) will cause elevation of endogenous neurotransmitter, which will in turn occupy more binding sites. As a result, fewer binding sites will remain available for binding by the labeled tracer, and the measured BP will be lower than at baseline. The fractional change in BP is the parameter that is most often reported as an indicator that there has been a prolonged change in neurotransmitter level (here, "prolonged" simply means on the order of, or longer than, the scan duration). Figure 4c illustrates the principle using the same scheme as in Figures 4a and 4b. Because specific binding sites exist in limited

number, the approach to full binding will follow a saturation curve. That is, for greater and greater amounts of neurotransmitter release, we expect to see less and less incremental reduction of binding potential.

Changes in Occupancy by an Exogenous Drug

A third common usage of PET and BP is for measuring occupancy of receptor sites by exogenous (unlabeled) drugs. This is a popular use of PET by pharmaceutical companies that typically want to know three things: a) does their candidate drug get into the brain; b) does the candidate drug hit the intended target; and c) what is the relationship between dose of the drug and percentage occupancy of the available (target) receptors? When companies are ready for a drug-occupancy study with PET, they usually already know the safe dose range of the drug (i.e., the range of doses that cause little to no adverse side effects). They also have a desired occupancy level in mind that will produce the desired drug effects. The question that PET can answer is: What is the receptor occupancy for each dose level in the allowable range? This relationship is characterized by an ED_{50} (drug dose at which 50 percent occupancy is achieved) and an E_{max} (maximal achievable level of binding if there were no upper limit on dose). Just as with elevation of endogenous neurotransmitter, the presence of a cold exogenous drug that binds to the same receptor as the tracer and reduces the concentration of available receptor sites can be imaged. This scenario is diagrammed in Figure 4d. An essential element of occupancy studies is that a tracer that binds selectively to the desired drug target must exist. On the other hand, the *drug* need not be selective. The change in binding of the PET tracer will reflect the occupancy of the drug only at the tracer's target. Again, occupancy of specific receptor binding sites is saturable, and reduction in BP (i.e., increase in drug occupancy) increases less and less for given increases in the drug as the concentration gets higher and higher. We typically define change in BP as a percentage change:

$$\Delta BP = [1 - BP(\text{under a challenge condition}) / BP(\text{at baseline})] * 100.$$

For the case of an exogenous drug binding to target sites, it turns out, Occupancy = ΔBP .

Ambiguities in Interpretation of PET Data

The flexibility of BP as an endpoint of PET studies with neuroreceptor ligands (as stated, one can measure receptors, transmitters, drugs) is also the source of potential ambiguity in interpretation. How can one tell the difference between lower receptor density under scan condition B vs. A from higher neurotransmitter level in scan condition B vs. A? These ambiguities are inherent in the compound parameter, BP. Generally, they can be resolved by considering the context of the measurement. If a stimulus was given just before the scan and the BP was lower than at baseline, we interpret this to mean that neurotransmitter levels rose due to the stimulus. We reject receptor up-regulation as the explanation, because it is a slower process than the time-scale of the PET scan (1 to 2 hours). On the other hand, if baseline scans are repeated on the same individuals after a year of psychotherapy and the average BP value is higher in the latter scan, we interpret this to mean that receptor number is increased by psychotherapy. (We must admit that long-term depression of baseline neurotransmitter level is also a valid interpretation.) Certainly we can say that "available receptor sites" were increased with therapy. In all cases, one must be alert to alternative interpretations of BP and ΔBP and try as best as possible to control for them via appropriate study designs.

Common Confounding Conditions in PET Experiments

Some sources of ambiguity in the interpretation of BP measurements are inherent in the nature of PET data, but others can and should be controlled experimentally.

Effect of Age. The densities of many neuroreceptors are known to decline with normal aging, and this has been confirmed

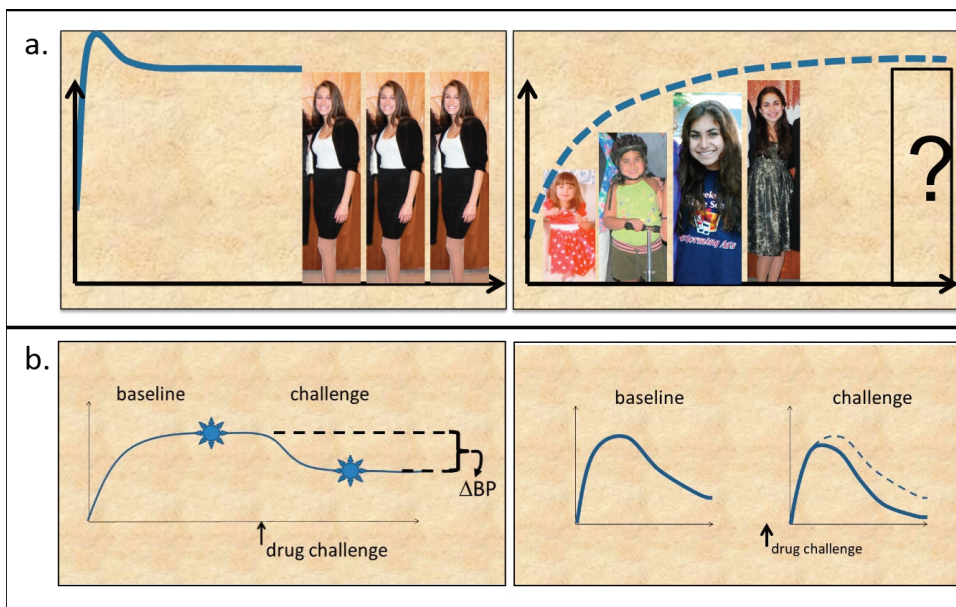


Figure 5. a) Schematic for (left) a type of steady state experiment for measuring height of a fully grown female child, as compared to (right) a type of non-steady experiment for predicting the adult height of the female children of one of the authors. **b)** Two common schemes for measuring change in BP with PET. General appearance of data from a paired bolus study (left) compared to a single bolus plus infusion study (right). Stars on right indicate that only two static measurements are necessary to get change in BP from an equilibrium study.

with PET [12]. Thus, BP will be lower in a group of healthy control subjects with a higher mean age than a second group whose mean age is younger. Similarly, there may be no effect of a treatment or condition (e.g., long-term drug abuse) on the numbers of a particular receptor, but it might appear so if the drug abusers have a mean age that is older than the mean age of the healthy subjects to whom they are being compared. Any careful reading of journal articles reporting BP values for different cohorts must include checking to make sure that the ages of the respective groups are not different. Similarly, a longitudinal study examining the effect of long-term treatment on a single group of individuals should correct for aging of the subjects if the length of the study is considerable.

Effect of Mass. As we saw above, an exogenous drug that occupies the target receptor reduces available binding sites for the tracer, and BP is reduced. This is the basis for drug occupancy studies. However, if the specific activity of the tracer (ratio of activity to mass) is low enough, then mass of cold tracer

acts like any exogenous drug. This poses two problems. First, we normally do not want the tracer species to exert its own drug effects. Second, the mass of cold tracer — as with any exogenous ligand for the target site — will occupy an appreciable number of receptors and the measured BP will be lower than if the mass of tracer were negligible. Unwanted drug effects notwithstanding, poorly controlled mass of tracer has the potential to introduce a confound into an experiment. If a patient group is being compared to a control group but the patients receive a significantly higher mean tracer mass (i.e., lower specific activity for the same amount of radioactivity injected), then the patients will appear to have lower BP due to their disease, when, in fact, the difference may be caused solely by a bias introduced by experimenters.

Experimental Approaches to Estimate Binding Potential

There are generally two approaches to estimating BP and, by extension, change in BP (Figure 5). Both approaches turn on recognizing that BP represents a steady state quantity

Table 1. Experimental design.

| Paired Bolus Design | Bolus Plus Infusion Design |
|---|--|
| Advantages | Advantages |
| Order of conditions can be randomized: baseline/challenge | Requires no model-fitting to estimate BP, Δ BP requires only one successful synthesis of tracer |
| Disadvantages | Disadvantages |
| Requires two successful syntheses | Requires computerized injection |
| Studies with [18F]-labeled tracers require two separate scan days; more chance of physiological variability | (High- and low-binding) regions don't all reach equilibrium at same time |
| | Requires that regions of interest reach equilibrium; data may be unusable if equilibrium is not achieved |
| | B/I scan needs more radioactivity than single bolus scan |

— the ratio of bound to free tracer in the tissue at steady state — that is, when the ratio of these quantities is not changing on a macroscopic level. To make such a measurement, one can either perform an experiment that brings the pools of bound and free tracer to steady state or if that is not possible or not desired, one can predict the steady state from non-steady measurements. If these ideas seem unintuitive, consider the two fun experiments depicted in Figure 5a for predicting the steady state (i.e., adult) height of one's daughters. The steady state approach means that one can make a few measurements (greater reliability than a single measurement) once the child reaches her adult height (Figure 5a, left). The non-steady approach means that one can make periodic measurements throughout childhood and — given a model of growth patterns of women in the United States — *predict* the adult height of the child based on these non-steady measurements (right).

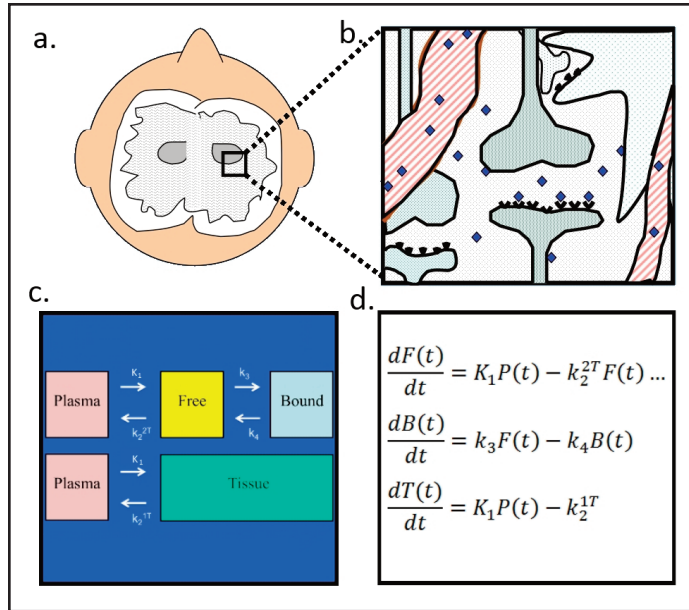
Bolus Plus Constant Infusion. In PET, the steady state or equilibrium approach to measuring BP consists of administering the tracer as an initial bolus followed by a constant infusion of additional tracer for the duration of the experiment. If the bolus and infusion fractions of the injection are balanced correctly, the TAC in the region(s) of interest will achieve a steady state in a minimal amount of time [13],

at which point tracer concentrations in plasma, free and bound compartments will remain in constant proportions to each other. At said point, BP can be measured directly from the levels of the plasma and tissue curves without the need for a model or any curve-fitting. It must be pointed out that infusions are more taxing experimentally. An infusion pump is required. More tracer is required (as compared to a bolus injection), since some of it decays while sitting in the syringe waiting to be infused. Not all tissue regions are the same. Tissue regions with differing kinetics of tracer uptake will reach equilibrium at different times — or not at all. Not all subjects are the same. For a given injection protocol, one subject's tissue curves might reach equilibrium but another's might not.

Bolus Studies. Alternatively, if an infusion experiment is impractical, a bolus administration of tracer is used. This approach includes a bolus injection of tracer, a dynamic acquisition of PET data, and a kinetic model to fit the data, estimate parameters, and calculate BP from the estimated model parameters. The parameters of the kinetic model are rate constants (they each have units of time^{-1}), but their ratio is an equilibrium (i.e., steady state) constant (BP is unitless).

Both experimental designs (bolus and bolus/infusion) can be used to measure the

Figure 6. Schematic of the tracer kinetic modeling process. **a)** Identify an organ of interest and a region of interest within it. **b)** Consider the relevant physiology or biochemistry. **c)** Abstract the tracer pools into connected compartments depending on the system (top-2T, bottom-1T). **d)** Write the mass balance equations.



change in BP. In the case of the bolus administration, two separate injections are required to measure change in BP (Δ BP) — perhaps in response to a drug challenge. A single bolus plus infusion (B/I) study can suffice to measure Δ BP, provided the drug challenge of interest acts rapidly enough and the tracer is sufficiently displaceable so that the effect can be detected during the duration of the scan. The two different paradigms for measuring Δ BP are diagrammed in Figure 5b. Table 1 details each paradigm's advantages and disadvantages that the investigator must consider carefully when planning a study. The order of a paired bolus study (baseline vs. challenge condition) can be randomized; the B/I cannot. Both scans of a paired bolus studies with ^{18}F -labeled tracers cannot both be performed on a single day. This may lead to greater variability in the data or even loss of some subjects who fail to return for a second scan. Equilibrium must be reached before the drug challenge in the B/I design. Unfortunately, there is no way of knowing that equilibrium has been achieved in a subject before giving the drug challenge, since PET data are not reconstructed and analyzed in real time. Finally, on the side of the B/I paradigm, the analysis of the data — provided equilibrium has been reached — is simple and requires no modeling and no curve fitting. For bolus studies, with some rare ex-

ceptions, one must use a kinetic model to describe the data in order to estimate BP.

Modeling Basics (To Get to Binding Potential via Bolus or Bolus + Infusion)

As we discussed earlier and diagrammed in Figure 3, the PET signal consists of tracer molecules in different pools, only one of which is the specific binding we are most interested in. These pools or compartments differentiate themselves over time. They have different temporal characteristics based on their degree of retention of the tracer. The PET signal can be dissected into its constituent parts with the use of a kinetic model that describes the processes of uptake and retention of the tracer, as well as the interconnectedness of the compartments.

The Modeling Process. The process of moving from some knowledge of the system of interest to a tracer kinetic model is diagrammed in Figure 6. One must first identify the organ(s) of interest. In the case of imaging drugs, the organ, often, is the brain. Next, one must consider the relevant (neuro)chemistry of the selected organ and how it relates to the tracer to be used. In a simple conception of the brain, we must include the vasculature that delivers the tracer to the tissue. How does the tracer traverse the blood brain barrier? Once inside the tissue, are there re-

ceptors or transporters to (specifically) bind the tracer? If there are multiple possible specific binding sites, is there one site that is likely to dominate? Inevitably, there will be nonspecific (i.e., non-displaceable) binding as well, because there are other entities in the tissue that appear to retain foreign molecules. Due to mathematical limitations (related to the limits of parameter identifiability), most models will treat the nonspecific binding pool as a sub-pool of the free, unbound tracer; nevertheless, we must keep in mind that such a process lurks under the surface even if it is not explicated in the model statement. Next, we must conceptualize the possible fates of the tracer into distinct pools or compartments of the model (all compartments are pools, but not all pools are compartments — see next section for explanation). Every route by which tracer can move from one compartment to another must be assigned a rate constant (designated by an arrow in Figure 6c). Finally, we turn a diagram of connected pools into a series of equations. Because what drives movement of tracer is mass action (e.g., diffusion from pools of high concentration to low), we must write mass balance equations for each compartment. Mass balance equations assert that the net accumulation of tracer over time is equal to the amount of tracer coming into the compartment per time, minus the amount of tracer leaving per time, plus tracer generated, minus tracer destroyed. Typically, generation does not apply. Our bodies do not create exogenous compounds. These equations take the form of ordinary differential equations. The only dependent variable is time. The dependent, or “state,” variables are the unknown concentrations in the respective compartments.

Compartmental Models (1T, 2T).

Compartments represent the unknown variables of a model (free tracer, F , bound tracer, B). These are sometimes referred to as “state” variables. Although in most circumstances plasma-borne tracer can be thought of as a distinct “pool,” we typically do not assign it a compartment, because it is measured directly via an arterial catheter and is therefore not an unknown. Rather, the

plasma tracer concentration over time is an input to the system. That is the case for the two most common compartmental models used to describe PET tracers: the one-tissue compartment (1T) and the two-tissue compartment (2T) models (see Figure 6c). Each of these models requires measurement of the arterial plasma concentration of tracer as the input function. Arterial blood taken from the arm is considered a good representation of the tracer concentration in arterial blood reaching the brain at each moment in time. For tracers that are known to bind specifically to a target, it would seem natural to model them with the 2T model. However, the 2T model has four unknown parameters: K_1 , k_2 , k_3 , k_4 . By contrast, the 1T model has one variable, the concentration of tracer in the tissue, and only two parameters, K_1 and k_2^{1T} . Note that the k_2 parameters have different meanings for each of the two models, and so in this chapter, we give them different superscripts to distinguish between them (the reader is advised that this is typically not done in the PET literature). While the 2T model would seem the intuitive choice — especially if we know that specific binding of tracer to a target occurs — it is not always supported by the data. That is, the specific binding may be too fast to allow for reliable estimation of k_3 and k_4 , or it represents only a small fraction of the total uptake, or perhaps the signal-to-noise ratio of the data is poor. Whatever the reason, if we cannot uniquely identify all the parameters of the 2T model by fitting it to the data, the 1T model can be used and the total volume of distribution, $V_t = K_1/k_2^{1T}$, becomes the estimated endpoint. By contrast, V_t as measured with the 2T model is defined as $V_t = K_1/k_2^{2T}(1 + BP)$. If the V_t is estimated from parameters of the 1T model, but specific binding exists, then k_2^{2T} implicitly contains effects of the specific binding term, BP .

Graphical Methods. To fit TACs with the 1T or 2T models requires an iterative algorithm and some knowledge of numerical methods, parameter estimation, and computer programming. There is a popular alternative to iterative curve fitting that can be used in many circumstances. Collec-

tively, these methods are based on rearrangements of the model equations to yield linear relationships between measured quantities [14-19]. One can think about these methods as transformations akin to a logarithm that transforms an exponential relationship into a linear one. The Logan plot was the first linearization of the 2T model to be applied widely to reversibly bound tracers (e.g., [^{11}C]raclopride). The slope of the original Logan plot is equivalent to the volume of distribution, V_t , the same parameter that can be estimated directly with either the 1T or 2T model [16]. An advantage of using the Logan plot is that it is possible to perform all the necessary calculations in a spreadsheet. Further, the estimate of V_t via the Logan plot is highly robust. That is, it almost never fails to produce an estimate with high precision. A disadvantage of the Logan plot is that it is not unbiased. It has been shown to underestimate V_t with increasing noise in the PET data [20]. As with proper experimental design, one must be cognizant of potential biases that can be introduced into the analysis by the model or the model transform and guard against misinterpretation.

Reference Region Methods. From the diagrams in Figure 6c, it would appear that one always needs a measured plasma input function to drive a kinetic model. On its face, this makes sense, since tracers enter (are inputted) into the system via the plasma. In fact, models designed to describe the data in the tissue can also work with input functions derived from reference regions in the image. A reference region is one that is essentially equivalent to the target region except that it is devoid of specific binding sites. By taking advantage of the fact that the same plasma concentration of tracer supplies both the target and the reference regions, it is possible to eliminate the plasma concentration from the model and describe the concentration in the target region compartments in terms of the reference region concentration. In effect, the reference region has become the input function. This concept was first applied to PET data by Farde et al. and Cunningham et al. [21,22]. Subsequent as-

sumptions applied by Lammertsma and Hume reduced the number of parameters in the reference tissue model (thus named the "simplified reference tissue model" (SRTM)) [23,24]. Finally, Gunn et al. devised an implementation of SRTM (using basis functions) that turned it into a linear model and thus almost as easy to use in practice as the Logan plot [25].

PET IMAGING OF DRUG CHALLENGE STUDIES

Here, we discuss conditions that may lead to biases in the use of PET to image receptor changes and drug-induced changes in the human brain. These themes will be repeated throughout the remainder of the paper as they arise in the discussions of the literature.

Novelty

Many different imaging groups measure drug-induced changes in dopamine release in the scanner or during the study day. However, dopamine is released in response not just to drugs of abuse but also to stress and to novelty. As the majority of subjects in these studies will not have been exposed to these experimental situations in their past, the experience will be novel to them. Suffice to say, it would not be helpful to be imaging novelty-induced dopamine release when one is trying to measure the effect of a drug. One way to avoid this common confound is to expose the subject to the study environment before their participation begins. In the case of our smoking-in-the-scanner studies, we have the subjects lie down in the scanner and simulate smoking at a session prior to a real scan session.

Order Effect

Order effects can occur in any scientific study. In rodent studies of drug treatment, when a placebo is compared to an active drug, the conditions are counter-balanced so that some rats receive the drug first and other rats receive the placebo first. This eliminates bias that could occur if the order in which drugs were given were to alter the results. In imaging stud-

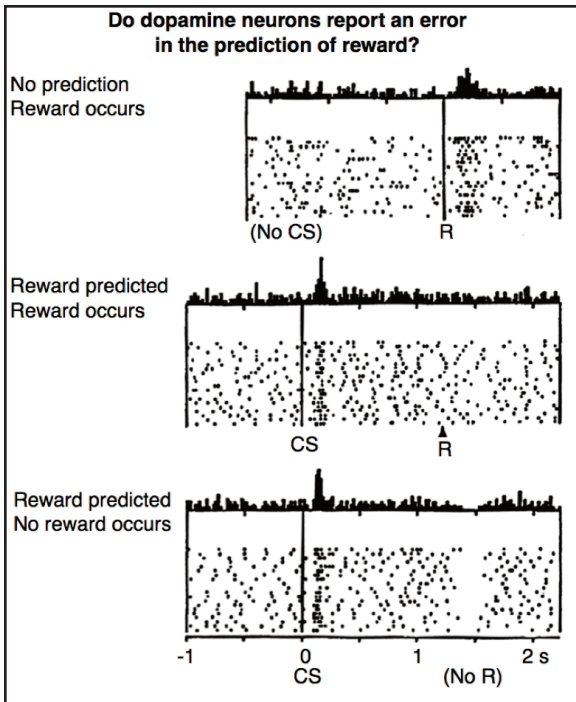


Figure 7. (Top) Prior to conditioning, reward without prediction causes a positive error in reward prediction, which increases DA neuron firing. (Middle) Following conditioning, the CS predicts the reward, leading to no prediction error. CS but no reward shows increase in DA neuron firing. (Bottom) After conditioning, CS but no reward causes a negative error in reward prediction. The CS causes increase in DA neuron firing, but the lack of reward causes a decrease in DA firing [26].

ies, this can be difficult logistically. When using radiotracers with short half-lives (carbon-11 has a 20.3 minute half-life), it is possible and sometimes preferable to do baseline and drug-challenge scans on the same day. This can reduce the variation between scans that may occur if scans are conducted far apart in time. It also increases the likelihood that the subject will be able to easily complete the study (e.g., it is usually easier for a subject to commit to one day at the PET center rather than having to take off multiple days from their job or school). However, this also makes it more difficult to randomize the order of scans.

Consider scans of amphetamine-induced dopamine release. Amphetamine's effect on dopamine (and thus ^{11}C -raclopride binding) is profound and long-lasting. It is not possible to do the drug-challenge scan on the same day before the baseline scan, since the effect of amphetamine would persist for hours (possibly longer) and corrupt a subsequent "baseline" measurement. On the other hand, if effects of a drug or other stimulus are short-lived, it is generally possible to counter-balance the scans.

Expectation and Reward-Prediction Error

We can learn from the work of Schultz and colleagues that dopamine neurons not only are activated in the presence of most drugs of abuse but that they are activated even before delivery of a drug, in response to cues and other stimuli that are "conditioned" or a conditioned stimulus (CS) [26]. Additionally, the dopamine neurons are sensitive to changes and errors in reward, which can be called prediction error. Dopamine neurons in the nonhuman primate brain fired after presentation of a reward that was not paired with a CS. When the reward and CS were paired, the dopamine neurons fired in response to the presentation of the CS and not to the subsequent presentation of the reward (Figure 7). That is, the dopamine neurons activated to the CS itself, because it was *predictive* of a reward [27,28]. When the CS is presented and then the reward does not occur (negative prediction error), there is the typical activation to the CS, but then a dip in dopamine neuron activation when the expected reward does not occur. This study highlights how sensitive the dopamine system is to cues and expectation of reward,

and care needs to be taken to design PET studies that take this sensitivity into account.

Sex Differences

Sex differences are evident in many psychiatric disorders, medical disorders, and also in the normal human brain [29]. There are sex differences in structure (e.g., total volume of the human brain and some subdivisions), in function (e.g., emotional processing as measured with fMRI), and in chemistry (as measured with PET). These differences are important to measure, as they may clarify the clinical literature. It might be helpful, for instance, to know if the higher prevalence of depression in women vs. men can be explained by greater serotonergic dysfunction in women. Unfortunately, sex differences can also cloud the interpretation of data — if they are not carefully recognized and controlled. In one of our own studies, we were at first convinced of differences in nicotinic acetylcholine receptor (nAChR) availability between healthy men and women when looking at a standard imaging outcome measure, volume of distribution (V_T). On further examination, however, we also found significant differences in total parent of the radiotracer (total unmetabolized radiotracer in the blood) and in f_p (the fraction of radiotracer free in the blood and not bound to plasma proteins). When these two factors were included in the analysis (by use of the normalized outcome, V_T/f_p), the apparent sex difference disappeared [30].

IMAGING DOPAMINE RELEASE IN RESPONSE TO ALCOHOL IN HUMANS

As suggested by microdialysis studies, the mesolimbic DA system is important as the common pathway through which all drugs of abuse — and other rewarding inputs — are processed. With PET and a dopaminergic ligand that competes with DA for binding to a receptor, one could, in theory, image the effects of reward processing in the brain in response to an addictive substance or behavior. In practice, such studies

have proved quite difficult, and the reader must take care to consider the strengths and weaknesses of each attempt. One issue is that alcohol does not cause a large elevation of DA levels above baseline — probably only a doubling or tripling of baseline. And, the effects of drinking are short-lived. A third challenge is that self-administration of alcohol by a volunteer (actually drinking a beer) is not easily performed within the confines of the PET scanner.

Imaging Dopamine Release in Response to Alcohol

If smoking is difficult for subjects lying in the scanner, it is nevertheless possible. Drinking while lying in the scanner is not. Researchers have taken two approaches to this problem. The most obvious study design for measuring alcohol-induced DA release is for the subject to drink an alcoholic beverage shortly before being scanned and compare raclopride BP in this condition to either baseline or placebo. This is the approach that was employed by the three studies to look at drinking-induced DA release, per se. Salonen et al., Boileau et al., and Urban et al. each used two-scan designs [31-33]. In each case, the conditions were either juice or alcohol plus juice. The conditions were randomized in the latter two studies. The Boileau and Salonen studies each consisted of six subjects; Urban scanned 11 men and 10 women. The subjects were social drinkers. The dose of alcohol was approximately 1 ml/kg in Boileau and Urban but about 1.3 ml/kg in Salonen. The blood alcohol levels in each study were consistent and generally reflected the respective doses (measurements were not taken at the same times, so direct comparisons are difficult). In any case, Urban claims that the dose in her study was equivalent to three standard drinks. Where the paradigms begin to diverge is the relative timing of alcohol and tracer. The alcohol was taken either 60 minutes, 30 minutes, or 5 minutes prior to tracer injection in Salonen, Boileau, and Urban, respectively. A second difference was that in the Urban study, the rim of the juice glass for the juice-only condition was coated with

Table 2. Summary of alcohol studies' experimental designs and outcomes.

| | Salonen (1997) | Boileau (2003) | Urban (2010) | Yoder (2009) | Oberlin (2013) |
|-------------------------|--|--|--|---|---|
| Tracer | [¹¹ C]raclopride | [¹¹ C]raclopride | [¹¹ C]raclopride | [¹¹ C]raclopride | [¹¹ C]raclopride |
| Injection | Bolus | Bolus | Bolus + infusion | Bolus | Bolus |
| Tracer dose | 2.89 – 3.51 mCi | 10 mCi | ~ 7.8 mCi | 14.1 ± 0.99 mCi | 14.9 ± 0.10 mCi |
| Scanner (resolution) | ECAT 931 (6.1 X 6.7 mm) | ECAT HR+ (4.8 x 4.8 x 5.6 mm FWHM) | ECAT EXACT HR+ | EXACT HR+ (9 mm FWHM) | EXACT HR+ (9 mm FWHM effective res.) |
| Design | Intra-subject (2 scans) | Intra-subject (2 scans) | Intra-subject (2 scans) | Intra-subject (3 scans) | Intra-subject (2 scans) |
| Phenomenon to test | Acute alcohol effect on DA release in the striatum | Alcohol induced DA release | Sex differences in DA release post alcohol challenge | Alcohol & alcohol cues | Beer flavor induced DA release |
| Subjects | 7 healthy, right-handed men | 6 healthy male nonalcoholics | 21 healthy men and women | 8 healthy subjects (5 male, 3 female) | 49 healthy male drinkers |
| Subject characteristics | Non drug or alcohol dependent | Nonalcoholic moderate drinkers | Nonalcoholic, 10-15 drinks/wk | Non drug or alcohol dependent; 2 FH+; 5 surpassed hazardous drinking threshold | Non drug/alcohol dependent, except 4 meeting DSM-IV for AD; 12 FH+ |
| Time from last drink | ~12 hrs | 24 hours | Since night before | ~24 hours | ~48 hours |
| Protocol | Drink BEFORE scan; 2 scans: 1) placebo, 2 hr break, 2) ethanol | Drink BEFORE scan, not told content of drink until this time | Scan order randomized | 3 scans: 1) neutral cues/no alcohol, 2) alcohol cues predict alcohol (but infusion delayed to post scan), 3) neutral cues with unexpected alcohol (infused during scan) | 2 scans, counter-balanced: 1) preferred beer flavor, 2) Gatorade® flavor |
| Timing | 3 separate drinks of placebo (75, 65, 55 min pre bolus), bolus, scan; 2 hr break; same schedule except using ethanol | Drink for 15 min, 30 min prior to bolus | Drink for 5-10 min, 5 min prior to bolus + infusion | Neutral or alcohol cues start 2 min after bolus, maintained 15 min | Beer or Gatorade flavor sprays (~15 ml) start 2 min after bolus, maintained 15 min |
| Alcohol administration | Self-admin | Self-admin | Self-admin | Investigator (IV infusion) | N/A |
| Alcohol type | Orange juice plus either tap water or ethanol | Orange juice with or without alcohol | Cranberry & soda with alcohol (~3 drinks worth) or trace alcohol | Ringer's lactate with or without alcohol | N/A |
| Ventral striatum | | ΔBP = 16.8 ± 16.3% | ΔBP = • Men: -12.1 ± 8% • Women: -6.2 ± 8% | • Cue condition w/ expected intoxication: -0.20 ± 0.1 • Unexpected alcohol condition: 0.12 ± 0.08 | ΔBP = • R ventral striatum: • FH+: 11.7 ± 4.1% (SE) • FH (ambig.): 3.8 ± 2.5% • FH-: 2.7 ± 2.7% |

Table 2. Summary of alcohol studies' experimental designs and outcomes, continued.

| | Salonen (1997) | Boileau (2003) | Urban (2010) | Yoder (2009) | Oberlin (2013) |
|----------|--|--|--|--|---|
| N. acc. | | Δ BP = 15.0 \pm 15.9% | | | |
| Putamen | Difference from alcohol to control: | Δ BP = 5.2 \pm 17.5% Ven- tral 13.7 \pm 17.5% | | | |
| Caudate | -0.10 \pm 0.12 BP (P = 0.43) | Δ BP = 4.0 \pm 16.4% | | | |
| Concerns | <ul style="list-style-type: none"> •Alcohol taken long time prior to scan. • Ethanol condition aversive to subjects? (1 subject too nauseous to have > 1 drink) | <ul style="list-style-type: none"> •Alcohol taken long time prior to scan. • Subjects drank large amount of alcohol – may have been aversive | <ul style="list-style-type: none"> • Biased to find greater ΔBP: control condition (smelling alcohol but not receiving any) may have caused negative prediction error | <ul style="list-style-type: none"> • No conditioning to cues (ala Schultz et al 997). Study assumes that cues are salient | <ul style="list-style-type: none"> • Only FH+ subjects showed effect of beer flavor on DA • Lack of resting baseline makes definitive determination of effect direction difficult |

alcohol to minimize any difference in the subjects' expectation between conditions. A final noteworthy difference is that the Urban study used paired bolus plus infusion scans, whereas the other studies both used the more standard paired-bolus design (Table 2).

The Salonen and Urban studies were essentially negative. No statistically significant decrease in [¹¹C]raclopride binding was found in any region of the striatum in the alcohol condition compared to juice. Boileau reported decreases of 14 to 15 percent in raclopride BP in regions that they termed Nucleus Accumbens and Ventral Putamen. Despite finding no statistically significant drop in BP in any individual region, Urban reported a sex difference in decrease in BP in all regions taken together [33]. What is clear from these studies is that imaging effects of oral alcohol on DA in the striatum is not easy and that we may be at the limits of detectability for raclopride-PET. Perhaps if we scrutinize elements of each design, we may find something worth tweaking. In all studies, the amount of alcohol was quite large. Some subjects — even social drinkers — may have found it aversive. Each study reported at least one subject who dropped out. As with smoking studies, the idea is to image drug-taking, not aversion. In the Salonen study, the alcohol was taken a lengthy time before the tracer (from 75 to 55 minutes before). Although the authors were careful to document considerably elevated

blood alcohol at both the beginning and end of the (1 hour) scanning period, one should not confuse elevated alcohol level with elevated DA. In fact, it is likely that DA responds early to drinking — possibly to the cues or the rapid rise in brain alcohol and then returns to baseline more quickly than alcohol level itself. In fact, it appears that Oberlin et al. have demonstrated that DA responds to the cues for alcohol rather than the alcohol itself [34]. Consider nicotine, which remains in the brain for days. Nonetheless, PET studies of cigarette smoking have detected only relatively brief DA elevation [35,36]. All three alcohol-drinking designs suffer from lack of a baseline condition. Juice alone is used as a placebo, but if the taste of juice is rewarding then perhaps DA was released in this condition. There is always a difficulty interpreting any study that contains only placebo and drug. The difference could be due to changes during drug or changes during placebo. Consider the Urban design [33]. In order to control expectation across conditions, the juice-only glass was rimmed with alcohol. But what if the smell of alcohol actually set up an expectation of alcohol? In that case, the juice condition was scanned while the subject experienced disappointment over not receiving the reward. This is called “reward prediction error,” and in monkeys, it has been shown to be the cause of decreased dopaminergic firing rates as discussed previously [26].

Table 3. Experimental designs and results of Weerts opioid receptor studies' of alcohol dependence.

| | Weerts (2008) | Weerts (2011) |
|--------------------------------|---|--|
| Tracer | [¹¹ C]carfentanil & [¹¹ C]methyl naltrindole | [¹¹ C]carfentanil & [¹¹ C]methyl naltrindole |
| Injection protocol | Bolus | Bolus |
| Tracer dose | [¹¹ C]CAR (19.4 ± 2.1 mCi); [¹¹ C]MeNTI (19.2 ± 3.2 mCi) | Avg. mCi: [¹¹ C]CFN: 19.30 (AD), 19.99 (HC); [¹¹ C]MeNTL: 18.87 (AD), 17.52 (HC) |
| Scanner (resolution) | GE (2 x 2 x 4.25 mm) | GE (5.5 x 6.1 mm FWHM) |
| Design | Inter-subject | Inter-subject |
| Phenomenon to test | Naltrexone occupancy of δ- and μ-opioid receptors | δ- and μ- opioid receptor availability at baseline |
| Subjects | 21 alcohol dependent & healthy control (15 male, 6 female) | 25 alcohol dependent & 30 healthy control |
| Subject characteristics | 60+ drinks/month, at least 5 drinks/occasion weekly | DSM-IV criteria for alcohol dependence; controls <8 drinks/wk women, <15 for men |
| Time from last drink | 15 days prior to naltrexone treatment | 5 days |
| Protocol | 15 days abstinence, followed by 4 days naltrexone | 2 PET scans in fixed order on the same day: [¹¹ C]MeNTL followed by [¹¹ C]CFN |
| Timing | 19 days inpatient, 50 mg p.o. 2x on day 15, then 1x daily for remainder of days; scan day | 5 days inpatient protocol; scans on day 5 |
| Mode of alcohol administration | N/A | N/A |
| Alcohol type | N/A | N/A |
| Ventral striatum | All ROIs: [¹¹ C]CAR: 94.9 + 4.9% occupancy | • BPND (AD) = 1.826 ± 0.068 • BPND (HC) = 1.438 ± 0.061 |
| Putamen | [¹¹ C]MeNTI: 21.1 + 14.49% occupancy | • BPND (AD) = 1.272 ± 0.044 • BPND (HC) = 0.962 ± 0.040 |
| Caudate | | • BPND (AD) = 1.395 ± 0.057 • BPND (HC) = 1.113 ± 0.052 |

Is there any evidence that reward prediction error leads to alteration of dopamine levels in humans? We must consider one last raclopride-PET paper to answer that question.

Yoder et al. took a different experimental approach to the study of alcohol-induced DA release [37]. Wary of the variability in alcohol absorption among people, they chose to administer the alcohol intravenously. The technique, called the “alcohol clamp,” was developed by O’Connor et al. and is based

on pharmacokinetic modeling of a variable-rate infusion of alcohol to maintain a constant blood alcohol level in each subject based on their height, weight, and gender [38]. Second, expectation was controlled through visual and olfactory cues, which preceded — and predicted — the delivery of alcohol or saline via IV. There were three conditions scanned in three separate sessions — each scanned with a bolus of [¹¹C]raclopride. The conditions were as follows: 1)

neutral cues signaling IV saline; 2) alcohol cues signifying alcohol; and 3) neutral cues coupled with unexpected alcohol. This design was constructed to decouple expectation from consumption of alcohol. The alcohol in condition 2 was delivered *after* the data acquisition was complete, so the condition can be thought of as “expectation of alcohol but no alcohol consumption,” whereas condition 3 — thanks to a little trickery — can be thought of as “alcohol consumption with no expectation of alcohol.” Subjects’ answers to questionnaires during the scan confirmed that expectations were controlled as intended. The results were quite provocative: In left ventral striatum, BP of “alcohol consumption without expectation” went down relative to condition 1. That is, this comparison signifies increase in DA. But, in the contralateral ventral striatum, BP of “expectation of alcohol without alcohol consumption” was *higher* than condition 1. The authors explained this combination of results as follows: Expectation of reward without reward is equivalent to negative reward prediction error. Reward without expectation of reward is positive prediction error. The caveat in this interpretation is that the subjects (heavy drinkers) must be considered to have been “conditioned” by their drinking history to respond with appropriate expectation to the cues. Accepting the author’s interpretation, this paper highlights the importance of controlling expectation in the study of drugs of abuse. To return to the Urban design [33], if the initial smell of alcohol on the rim of the juice-only glass was a cue for imminent alcohol reward, then we might expect DA to decrease during this scan. When compared to a second condition (i.e., alcohol drink), any apparent increase in DA with alcohol could, in fact, be the result of decrease in the juice-only condition.

Imaging the Opioid Receptor System in Response to Alcohol and Treatments for Alcoholism

There are many other important lines of investigation of alcohol abuse using PET other than examining DA release. Some have looked at receptor number [39,40], and

others have looked at change in BP due to drugs for treatment of alcohol. We highlight two studies, briefly (Table 3). Both studies are by Weerts et al. [41,42]. Both studies looked at the Mu opioid receptor (MOR) and the Delta opioid receptor (DOR), thanks to the use of two selective tracers, [¹¹C]carfentail and [¹¹C]methyl-naltrindol. Collectively, the two studies looked at baseline levels of MOR and DOR, as well as occupancy of these receptors due to four days of treatment with naltrexone (NTX), a non-selective opioid receptor antagonist that is prescribed for alcohol abuse. Baseline receptors in alcoholics were compared to healthy controls, and occupancy of naltrexone was measured in alcoholics only. The main findings were that clinical doses of naltrexone occupied 95 percent of MOR but only ~21 percent of DOR. Second, MOR and DOR levels in high binding regions of the brain were higher in alcoholics than in controls (differences for MOR were significant; DOR were not). The first finding suggests that any variability in efficacy of NTX for helping alcoholics to stop drinking is probably not mediated by binding to MOR, since all alcoholics were uniformly blocked. The second finding suggests that years of drinking may lead to upregulation of MOR and DOR, which is more like the behavior of nicotinic acetylcholine receptor [43] and less like DA D₂ receptors [39].

Development of new pharmacotherapeutic approaches to treating alcohol dependence should be based on our understanding of the behavioral and neurochemical mechanisms mediating alcohol drinking, as well the efficacy of agents known to reduce alcohol drinking. Clinical trials indicate that treatment-seeking heavy drinkers who receive NTX at a dose of 50 mg/day [44,45] or 100 mg/day [46] have lower levels of relapse to drinking during the treatment period than do those receiving placebo. Subpopulations of alcohol-dependent patients may respond better to NTX [47], and family history (FH) of alcoholism is emerging as an important predictor of NTX response [48,49]. Developing a better pharmacological understanding of this dif-

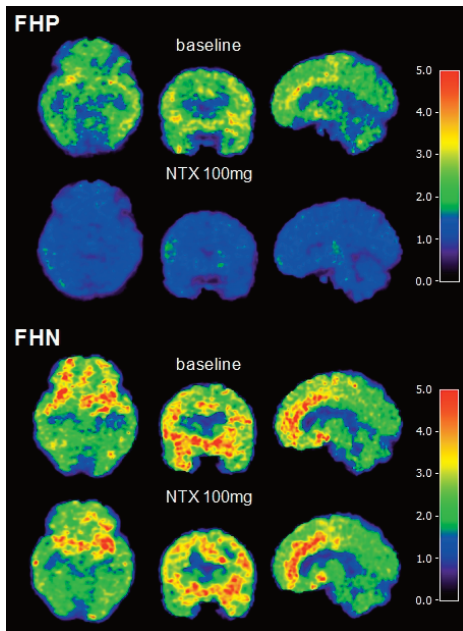


Figure 8. Occupancy of NTX at KOR in heavy drinkers as illustrated by V_T images at baseline and after a week of treatment. Degree of reduction in V_T after a week of NTX treatment indicates degree of NTX occupancy at KOR. Dramatic decrease in V_T in an FHP drinker (compare top two rows) indicates high (>90 percent) NTX occupancy, whereas little change in BP (compare bottom two rows) indicates low NTX occupancy in FHN. FHP = Family History Positive for alcoholism, FHN = Family History Negative for alcoholism.

ferential response could help enhance the efficacy of NTX and other treatments.

Emerging evidence suggests an important, but complex, role for the Kappa Opioid Receptor (KOR) system in alcohol drinking. Dynorphin is the endogenous opioid ligand that binds to both MOR and KOR [50]. In general, activation of KOR is aversive. Animals will not self-administer dynorphin, administration of dynorphin results in conditioned place aversion in animals, and dynorphin activation of KOR results in decreased DA release in brain reward areas [51,52]. The potential role of KOR in mediating alcohol drinking behavior has been the focus of several studies in animals and humans. Mice that lack the KOR drink more alcohol and have greater release of DA in response to alcohol [53]. Rodents with an in-

creased propensity to consume alcohol have lower dynorphin levels in several brain regions that are involved in the control of alcohol drinking [54-56]. In humans, the presence of the OPRK1 allele (which decreases expression of KOR) is associated with an increased risk of alcoholism [57]. The above evidence suggests that differences in the status of the KOR system may mediate differences in propensity to drink alcohol.

Work in Progress

Naltrexone is a non-specific opioid antagonist that binds dose-dependently to all three sub-types of opioid receptors (MOR, DOR, and KOR). It has been shown to be efficacious in the treatment of alcoholism [44]. To date, it has not been possible to use PET to probe the binding of naltrexone to KOR for a very simple reason. There had not been a reliable KOR-selective PET tracer until recently, when an antagonist tracer for the KOR was developed [58]. The tracer [^{11}C]-LY2975050 has been shown to be selective *in vivo* for the KOR [59]. With this new tracer in hand, we are presently working at Yale to measure the occupancy of NTX at KOR in the brains of alcoholics, to relate occupancy at KOR to measures of drinking behavior and to investigate the effects of long-term drinking on KOR availability. Preliminary results in a few alcohol-dependent subjects suggest that like binding to DOR, there may be considerable variability in occupancy of NTX at KOR (Figure 8) [60]. Through these efforts, we hope to complement the findings of Weerts et al., fill in missing gaps in our knowledge of NTX's action at multiple targets, and gain insight that may aid in development of the next generation of pharmacotherapies for alcoholism.

CONCLUSION

PET is a powerful imaging technique that is unique among high resolution functional imaging modalities for its molecular specificity. But even with its great strengths, PET must be used properly to produce valid and useful research results. The raw emis-

sion image (representing tracer concentration in the tissue) is almost never the end-product of interest. Kinetic modeling is the means by which PET data are converted to useful physiological parameters. A common endpoint, discussed in this review, is binding potential. It has many uses for the study of neuroreceptors, neurotransmitters, and exogenous drugs. Even with sophisticated modeling techniques, however, ambiguities (did receptors up-regulate or did neurotransmitter level decrease?) and biases (were the cohorts well-matched for age and mass of cold carrier?) may be hiding in the data. To make best use of PET, one must recognize the potential for said ambiguities and take pains to design experiments carefully so that ambiguities and biases are prevented or, at the very least, acknowledged. We have illustrated a few of the strengths and weakness of PET experiment design through the review and discussion of studies related to the brain's response to alcohol and medications for alcoholism.

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