Brain Imaging in Nonhuman Primates: Insights into Drug Addiction

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Abstract

In vivo brain imaging enables the systematic examination of trait and state variables that contribute to the etiology of human diseases. This review highlights the use of in vivo imaging in nonhuman primate models of drug abuse. In efforts to translate findings from laboratory animals to humans, monkey models offer considerable advantages over those that use rodents and other species because of their neurobiological similarity to humans and their longer life span, which makes it possible to study individual subjects over several years. This article provides a brief overview of positron emission tomography (PET), magnetic resonance imaging (MRI)–based techniques, and encephalographic approaches, with a focus on methodological issues that investigators new to the field should consider. We discuss PET imaging studies involving the dopamine (DA) system, with a special emphasis on DA D2 receptors, and describe experimental approaches through which PET imaging data can provide information about the neuropharmacological and neurochemical actions of drugs that modify behavior. We also consider the use of imaging to understand the impact and interactions of genetic predispositions and environmental and physiological modulators on disease states. For MRI-based and encephalographic studies, we describe approaches that can provide new information about brain function. Although much work remains to be done to adapt and apply these techniques for routine use in nonhuman primates, there has been much progress. These techniques will provide the foundation for future studies aimed at developing behavioral and pharmacological treatments for many human diseases.

Key Words: animal models; D2 receptors; dopamine; magnetic resonance imaging; nonhuman primates; positron emission tomography

Introduction

The goal of this review is twofold. First, we describe in vivo imaging techniques in nonhuman primates with the goal of providing some basic background for interested investigators. Second, we introduce readers both to the use of animal models for studying disease states, in this case drug addiction, and to uses of in vivo imaging for addressing new and exciting questions. We focus on models of drug abuse, but the research questions are relevant to all models of human disease.

With respect to imaging and nonhuman primates, as with any other research technique there are advantages and disadvantages. Using animal models scientists can perform experiments that are ethnically or practically impossible in humans—namely, imaging the brains of experimentally naïve individuals before drug exposure in order to assess trait variables (i.e., whether a brain measure represents a preexisting characteristic that is predictive of an outcome). Furthermore, it is possible to repeatedly image an animal throughout the experiment to determine whether a brain measure was changed by the independent variable (i.e., whether the independent variable was a state variable). As with all animal models, we can control many variables in order to better understand how one or a few affect brain function. This enhanced experimental control is, of course, advantageous, but it can also be a limitation: in studies on humans, a multitude of factors can affect the dependent variable in any given experiment; these interactions are clearly relevant and need to be investigated. The development of animal models and brain imaging methodologies continues to grow in sophistication such that future studies will minimize this limitation.

As research subjects in brain imaging studies of addiction, nonhuman primates offer several advantages over rodents and other laboratory animal species. First, abundant evidence indicates that rodent and primate brains differ in the anatomy, physiology, and neurochemistry of brain neurotransmitter systems that mediate the abuse-related effects of drugs, including the dopamine, norepinephrine, and opiate systems (Berger et al. 1991; Cragg et al. 2000; Haber and McFarland 1999; Joel and Weiner 2000; Monsour et al. 1988; Smith et al. 2006). Moreover, compared to rodents, monkeys are more similar to humans in the pharmacokinetics and metabolism of several drugs (Ward and Smith 2004a,b), including the mu opiate receptor antagonist naltrexone (Misra et al. 1976; Reuning et al. 1989), the sero-
tonin-releasing drug fenfluramine (Mennini et al. 1996), and 3,4-methylenedioxyamphetamine (MDMA; Bowyer et al. 2003; Chu et al. 1996; de la Torre et al. 2004; Banks et al. 2007). In addition, imaging data have shown that the nonhuman primate brain differs substantially from the rodent brain in terms of cocaine-induced changes in brain metabolism (Lyons et al. 1996; Porrino et al. 2002). A further advantage of monkey experiments is that SPECT studies use different radioactive substances across time. To quantify and compare image data, the investigator determines how the DVR changed (receptor number or concentration). These dependent variables are unitless numbers that represent the ratio of receptor density to affinity and the denominator (affinity for the receptor). Importantly, this is considered an advantage of PET because it provides a measure of receptor binding in the living brain. That is, receptor binding is assessed in the presence of endogenous neurotransmitters that can compete with the radioligand. Depending on the research question, one may wish to better determine the DVR (receptor number or changes in levels of the neurotransmitter). We address that issue below using dopamine (DA) as an example.

The principles of PET imaging that lead to its utility in studies of brain function are also characteristic of a related imaging technique, single photon emission computed tomography (SPECT). The primary difference between the techniques is that SPECT studies use different radioactive

**PET Imaging**

In vitro techniques such as receptor autoradiography have been effective for characterizing brain changes after chronic drug administration in nonhuman primates (see Porrino et al. 2004 for a review). More recently, the development of brain imaging techniques has enabled in vivo assessments of brain function in laboratory animals. In this section, we focus on one such technique, positron emission tomography (PET), which is perhaps the most frequently used imaging modality with nonhuman primates. We also briefly describe several other imaging modalities based on magnetic resonance imaging (MRI) and encephalographic techniques that can provide data that complement results of PET studies.

The basic descriptions below provide an introduction to imaging techniques. Studies that use these techniques require a large institutional investment; thus, an investigator would not add PET imaging to a research program if the institution did not already have a PET camera and cyclotron (as well as physicists, radiologists, radiochemists, and other qualified personnel). Readers can learn more about these techniques in textbooks by Senda and colleagues (2002) and by Huettel and colleagues (2003).

**Methodological Considerations**

**Experimental Parameters**

Positron emission tomography (PET) derives its name from its function. After a radioactive PET ligand is injected into a subject, decay of the ligand produces positrons, which travel in space in a random fashion until they collide with an electron. The resulting annihilation causes the emission of gamma ray photons that project at 180°. PET cameras have detectors arranged in a ring around the subject; the stimulation of two detectors (at 180°) reveals in 3D the location of the annihilation and thus of the radioactive molecule or tracer, producing a 2-dimensional image of a slice through the brain (i.e., tomography). The most frequently used radiotracers for PET studies contain \(^{15}\)O (with a half-life of 2 minutes; i.e., \(^{15}\)O water to assess blood flow), \(^{11}\)C (with a half-life of 20 minutes), and \(^{18}\)F (with a half-life of 110 minutes). The latter two are the most frequently used tracers for receptor ligands. \(^{18}\)F is also used to assess glucose metabolism using radiolabelled fluorodeoxyglucose (\([^{18}\text{F}]\text{FDG}\)).

When analyzing results, the investigator determines which areas of the brain to examine and uses MRI images to identify these regions of interest (ROIs) (Figure 1). Determining ROIs before examining the PET data enables an unbiased assessment of how an independent variable affected the PET measures in specific brain regions. After a PET study, the investigator superimposes the PET images on the MRI images and uses the ROIs to measure the tissue content of the radioactive molecule. For receptor ligand studies, the analysis of the PET data follows a three-compartment model: radioactivity in the blood, in the extracellular space in the brain, and bound to the receptor. Rates of movement of the tracer between compartments are analyzed to generate a distribution volume (DV) in the ROI, which serves as a measure of the percent injected dose of radioactive substance across time. To quantify and compare PET data after some manipulation, the DV for one ROI is studied in relation to a control region that contains relatively few receptors bound by that ligand. The ratio of these DVs constitutes the distribution volume ratio (DVR), which is the primary dependent variable in most PET imaging studies. A related dependent variable frequently reported is the binding potential (BP), defined as the DVR-1. These dependent variables are unitless numbers that represent the ratio of receptor density to affinity (i.e., Bmax/Kd) for a receptor. Thus, changes in DVR may result from changes in either the numerator (receptor density) or the denominator (affinity for the receptor). Importantly, this is considered an advantage of PET because it provides a measure of receptor binding in the living brain. That is, receptor binding is assessed in the presence of endogenous neurotransmitters that can compete with the radioligand. Depending on the research question, one may wish to better determine how the DVR changed (receptor number or changes in levels of the neurotransmitter). We address that issue below using dopamine (DA) as an example.

The principles of PET imaging that lead to its utility in studies of brain function are also characteristic of a related imaging technique, single photon emission computed tomography (SPECT). The primary difference between the techniques is that SPECT studies use different radioactive
substances with longer decay times. In addition, because SPECT radiotracers emit single photons rather than pairs, the technique is less sensitive than PET and the resulting images are less detailed. The primary advantage of SPECT versus PET is a lower expense and a relatively greater ease of implementation. For a thorough introduction to the use of SPECT, see relevant chapters in Kaufman (2001).

**Imaging Conscious versus Unconscious Subjects**

A main issue in imaging studies is whether the subject should be anesthetized or awake and behaving during the scanning protocol. We discuss examples of both types of studies in monkeys, but this independent variable naturally depends on the question under study. If anesthesia is used, the anesthetic agent may influence the dependent variable. The best way to address these questions is to use a within-subjects design and to compare data between the two studies.

Only a few research groups have published PET imaging work in awake monkeys. Howell and colleagues (2001) described a restraint apparatus that permitted the study of awake monkeys in the PET scanner. The apparatus was a modification of a standard Primate Products (Redwood, CA) restraint chair, in which a customized head holder mounted to the chair prevented head movements during the acquisition of PET data. The researchers acclimated the monkeys to the restraint apparatus over a 10- to 12-week period, so that the monkeys could remain in the apparatus for up to 4 hours per session, twice a week. Measures of plasma cortisol levels from these monkeys during immobilization indicated that by the end of training the restraint did not cause significantly elevated levels of cortisol (Howell et al. 2001).

In the first study using this apparatus, the effects of acute intravenous 1.0 mg/kg cocaine administration on cerebral blood flow were assessed using $^{15}$O-labelled water. Significant increases in blood flow induced by cocaine were evident in the whole brain, striatal regions, and medial temporal and frontal cortical regions. High test-retest reliability provided further evidence of the orderliness of these pharmacological effects. Additionally, replications of the experiment showed that pretreatment with the selective serotonin uptake inhibitor alaproclate blocked cocaine-induced increases in cerebral blood flow (Howell et al. 2002). The effects of cocaine on cerebral blood flow in these previously cocaine-naive monkeys contrast with reports describing cocaine-induced decreases in cerebral blood flow in humans with an extensive history of cocaine use (Kaufman et al. 1998a,b; Pearlson et al. 1993; Wallace et al. 1996).

Depending on the research question, it is not always necessary to use conscious, behaving monkeys in PET im-

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**Figure 1** Left panel: MRI from a monkey showing three planes (horizontal, coronal, and sagittal). Circles indicate regions of interest (ROIs). Right panel: Co-registered MRI and PET image following an $^{18}$F-FCP study. The original figure is in color and is available in the online posting of this article at www.ilarjournal.com.
aging studies. For example, our group has been interested in how environmental and pharmacological variables influence DA receptor availability. To address this, we have examined one of the DA receptor superfamilies, the D2-like receptor. Questions about D2 receptor availability as a trait or state variable do not require the monkey to be conscious during the PET study. However, it is essential to address issues related to anesthesia in order to ensure that effects on PET measures are due to independent variables and not to an interaction between those variables and anesthesia.

Investigators starting a research program that involves PET need to determine which anesthetic to use. The choice depends on several factors, including the duration of the PET scan and the radiotracer used. For our studies, which were approximately 3 hours long and in which most radiotracers were labelled with $^{18}$F, we chose isoflurane because of its minimal effects on neurotransmitters that can influence DA levels (Lecharny et al. 1995) and because other studies have shown that isoflurane had no effect on availability of the D2-selective radiotracer $^{[11]}$Craclopride (Tsukada et al. 1999). A reasonable question that has not yet been directly addressed is how blood flow changes over the course of a 3-hour PET study; additional basic PET research in monkeys is needed.

The impact of the anesthetic depends on the radioligand’s primary target. For example, if the ligand binds to opiate receptors, it is unlikely that an anesthetic that affects DA would produce significant effects on the PET measure. In contrast, that same anesthetic may affect measures of DA receptor availability. For example, the most frequently used anesthetic in veterinary medicine involving nonhuman primates is ketamine, which is generally the anesthetic of choice for immobilizing a monkey and transporting it to the PET center. However, ketamine affects levels of extracellular DA (Lindefors et al. 1997) and might therefore affect PET measures of DA receptor availability. In a PET study involving conscious rhesus monkeys, Onoe and colleagues (1994) found that a low dose of ketamine (5 mg/kg) administered 30 minutes before the D2 ligand $^{[11]}$CN-methylspiperone (NMSP) increased the binding of NMSP. In two studies with five experimentally naive monkeys we investigated whether induction with ketamine would affect our measures of D2 receptor availability using the ligand $^{[18]}$Ffluoroclopride (FCP) and a within-subjects design (Nader et al. 1999). In the first study, anesthesia was induced with 4% to 5% isoflurane and maintained with 1.5% isoflurane for the duration of the PET study. In the second study, using the same monkeys at least 1 month later, anesthesia was induced with 10 mg/kg ketamine and maintained with 1.5% isoflurane. We found an approximately 2% difference in D2 receptor availability between the two studies, indicating that ketamine-induced anesthesia did not significantly influence our measures of DA receptor availability.

To summarize this section, investigators need to collect pilot data before undertaking a long-term PET imaging project. If anesthesia is a potential issue, experiments should be conducted to compare awake monkeys with anesthetized animals. If the differences are not statistically significant, then it is probably not necessary to train the remaining experimental group(s) for conscious PET studies unless the experiment calls for PET imaging in behaving subjects. If anesthesia is to be used, it is important to consider the direct and indirect pharmacological effects of the agent on the primary PET target. Again, within-subjects, repeated-testing experiments will be necessary to determine the best anesthetic to use in these studies. Finally, changes in blood flow over the course of a PET study, whether the subject is conscious or unconscious, have not been examined in any study. This basic information needs to be a priority in future studies.

**Correlating Neurochemistry with Behavior**

As mentioned above, PET is an in vivo measure of brain activity that can provide valuable longitudinal data about ongoing behavior. PET can also be used to begin to noninvasively examine neurochemical mechanisms that underlie changes in both behavior and the PET signal. We describe two general applications in this section. The first entails administration during a PET study of nonradiolabelled (“cold”) compounds that can indirectly affect neurotransmitter levels to provide information about the interactions of the PET tracer with extracellular neurotransmitters. The second approach involves correlation of PET data with behavioral measures known to be sensitive to neurotransmitter levels in order to better understand the mechanisms that mediate behavior. Our goal in this section is to provide ideas for consideration in the development of research questions rather than to provide an exhaustive review of the methodology.

**Modifying D2 Receptor Availability by Changing DA Levels**

With the use of tracers that are receptor ligands, PET imaging data provide an index of receptor availability. That is, the dependent variable (DVR or BP) is affected not only by receptor density but also by extracellular levels of the neurotransmitter, which competes with the radiotracer. Additional experiments are necessary to determine whether the changes in DVR or BP are related to changes in receptor protein or extracellular neurotransmitter levels. Such experiments could involve in vitro receptor autoradiography to measure receptor densities or in vivo microdialysis to measure extracellular neurotransmitter levels. Thus, the major advantage of PET imaging—the ability to assess brain function in a living animal—brings with it some ambiguity about the neurobiological mechanisms that mediate group differences or changes in the PET signal in individual subjects over time. Fortunately, there are several approaches that can shed light on these underlying processes and aid in characterizing the neuropharmacological effects of drugs.
Several investigators have used PET in combination with other, nonradiolabelled drugs to understand how changes in neurotransmitter levels alter radiotracer binding (see Laruelle 2000 for review). Studies document that agonist-induced elevations of DA decrease measures of radioligand binding to postsynaptic DA receptors (e.g., Innis et al. 1992; Mach et al. 1997; Seneca et al. 2006; review by Laruelle 2000). For example, using SPECT, Innis and colleagues (1992) examined the effects of several pharmacological agents on D2 binding of the radiotracer [123I]iodobenzamide (IBZM) in baboons and rhesus monkeys. Increases in levels of DA, produced by administration of the indirect-acting DA agonist d-amphetamine, reduced IBZM binding, implicating displacement of the radiotracer by extracellular DA. To provide additional evidence for this proposed mechanism, two baboons were first treated with reserpine, an agent that has been shown to deplete endogenous DA, before the d-amphetamine experiment. In both animals, reserpine attenuated amphetamine-induced reductions in IBZM binding. After the animals were sacrificed, striatal DA levels were found to be 90% lower in reserpine-treated baboons compared to control animals, further supporting the hypothesized mechanisms of action. Understanding the degree to which the binding of a PET radiotracer is sensitive to changes in levels of extracellular neurotransmitter, coupled with knowledge of the relative affinity of the tracer and extracellular neurotransmitter for the target, can assist in interpreting the neurobiological causes of changes in DVRs over time.

Another strategy for using cold compounds with PET is to decrease the levels of neurotransmitter competing with the radiotracer. Using the DA D2 receptor ligand [11C]raclopride, Dewey and colleagues (1992) conducted two studies in the same animal on the same day to investigate GABA-mediated inhibition of DA release in baboons. The first PET study was a baseline [11C]raclopride scan; the second followed the administration of either the indirect-acting GABA agonist gamma-vinyl-GABA (GVG), an irreversible inhibitor of the enzyme that catabolizes GABA and subsequently increases GABA levels, or the benzodiazepine lorazepam, which increases the affinity of GABA for its receptor. If DA competes with [11C]raclopride for D2 receptors, decreasing levels of DA should increase the PET signal. Dewey and colleagues (1992) found that in certain brain structures, GVG and lorazepam administration increased D2 receptor availability by nearly 30% and 50%, respectively. These findings were consistent with the hypothesis that GABA agonists decreased DA levels and that the interaction between [11C]raclopride and D2 receptors was influenced by DA.

With this approach in mind, investigators interested in obtaining a measure of receptor levels less influenced by changes in neurotransmitter levels could determine baseline PET measures in the presence of a cold compound that decreases neurotransmitter levels and use this procedure longitudinally to document changes over time. Using [18F]FCP, which has similar binding properties to [11C]raclopride (Mach et al. 1993), we examined whether lorazepam altered D2 receptor availability and did not find any significant effects (Nader et al. 2006). However, the variability between and within subjects was lower when PET studies were preceded by lorazepam, so this protocol was adopted throughout the longitudinal experiment. Interestingly, when the effects of reinforcing doses of cocaine were examined over a 1-year period, the results were consistent with in vitro receptor autoradiography studies showing that cocaine exposure reduced D2 receptor levels (Nader et al. 2002). There was, however, one exception: 1 week after monkeys began self-administering cocaine, D2 receptor availability in the presence of lorazepam, which should have decreased DA levels, was significantly reduced, suggesting D2 receptor downregulation. However, in vitro receptor autoradiography in monkeys with similar cocaine histories did not indicate significantly lower D2 receptor densities compared to controls (Nader et al. 2002). Taken together, these results suggested that, even in the presence of lorazepam, extracellular DA levels influenced the PET measures.

One additional use of cold compounds in PET studies involves identification of receptor subtypes for which no radioligand is yet available. For example, in the D2-like superfamily of receptors, there are three subtypes: the D1, D2, and D3 receptors. Radiochemists have been working feverishly to develop a PET radiotracer for the D3 receptor (Newman et al. 2005), but no D3-selective radioligand has yet been validated. Compounds such as raclopride and FCP bind with similar affinity at D2 and D3 receptors, so they are said to provide information about the D2-like receptor superfamily, but not any particular subtype. In the field of cocaine abuse research, this is significant because cocaine appears to affect these receptor subtypes differently. For example, a large body of data suggests that chronic cocaine exposure decreases levels of D2-like receptors (e.g., Martinez et al. 2004; Nader et al. 2002, 2006; Volkow et al. 1996). Studies using cold D3-selective antagonists administered during PET studies with raclopride or FCP could provide valuable information about D2 and D3 receptor function as a consequence of cocaine exposure (or other independent variables). Although beyond the scope of this article, there are several pharmacological characteristics that make radioligands, including direct-acting agonists or antagonists, more or less suitable for displacement studies (Seneca et al. 2006).

Future studies involving cold compounds and measures of extracellular DA could incorporate more invasive techniques such as in vivo microdialysis (Bradberry 2000; Czoty et al. 2002; Howell and Wilcox 2002). Dewey and colleagues (1995) conducted one of the first studies to use both PET and in vivo microdialysis, acquiring PET data in baboons and microdialysis data in rodents. The study found that a serotonin agonist, citalopram, could increase D2 receptor availability in baboons as measured with PET and [11C]raclopride and that citalopram decreased extracellular DA in rats by approximately 50%, consistent with the hy-
Correlating Displacement of a PET Tracer with a Behavioral Outcome

Once it has been established that drug-induced changes in extracellular neurotransmitters can lead to displacement of a radiotracer, the next step is to associate the behavioral effects of the drug with its ability to alter radiotracer binding in order to correlate neurotransmitter activity with a behavioral outcome. For example, Volkow and colleagues (1999) compared the ability of methylphenidate (MP) to displace $[^{11}]$Craclopride binding with its subjective effects in healthy, non-drug-abusing subjects. There was a significant and positive correlation between the intensity of the subjective reports of “high” and the ability of MP to elevate DA, as assessed by displacement of $[^{11}]$Craclopride. Consistent with this finding, in the subjects that did not perceive a “high,” MP did not elevate DA. Howell and colleagues have extended the use of PET to examine relationships between receptor occupancy and the ability of drugs to decrease cocaine self-administration (e.g., Howell et al. 2007; Lindsey et al. 2004), and Heinz and colleagues (1998, 2003) have examined serotonin transporter availability, aggression, and alcohol intake in monkeys. These studies highlight a major strength of PET: it facilitates the study of brain function in behaving subjects. Such information will provide insight into novel treatment strategies for affective disorders.

With the knowledge that drugs that induce neurotransmitter elevations can alter PET measures, it is possible to design experiments to examine how other variables displace the radiotracer, thereby providing indirect evidence of the involvement of a neurotransmitter in a behavioral outcome. To highlight this point we briefly present two examples using within-subjects designs: one study investigated effects of environmental variables, and the other documented interactions between hormone levels and the PET signal.

For over a decade, our group has been interested in brain changes due to social housing in nonhuman primates. Among macaques (Old World monkeys), social rank is determined by the outcomes of social interactions, particularly fights. The hierarchy is linear and transitive (e.g., Kaplan et al. 1982). The first PET study of D2 receptor availability in socially housed monkeys focused on female cynomolgus monkeys that had lived in stable social groups for over 3 years. In that study, subordinate monkeys had a significantly lower average D2 receptor availability than dominant monkeys (Grant et al. 1998). It was hypothesized that this difference resulted from stress-induced elevation of DA levels in the subordinate monkeys.

We initiated a study that began with 20 experimentally naïve, individually housed male cynomolgus monkeys (Morgan et al. 2002). We were interested in two general questions: (1) Was D2 receptor availability a trait marker for eventual social rank, i.e., would PET measures predict (correlate with) eventual social rank? and (2) Would D2 receptor availability change as a result of social group formation, i.e., was it a state variable? The results were intriguing: D2 receptor availability was not a trait marker that predicted eventual social rank; however, it was a state variable that changed due to social hierarchy. We replicated the Grant et al. (1998) findings by showing that subordinate monkeys had significantly lower D2 receptor availability compared to dominant monkeys (Morgan et al. 2002). However, the mechanism for these differences was not what was hypothesized. Instead of the D2 measures decreasing in monkeys that became subordinate, as one would hypothesize if stress-induced elevation of DA were the mechanism, we found that D2 receptor availability was unchanged in monkeys that became subordinate but increased in monkeys that became dominant. We do not know whether the alteration in D2 availability resulted from a decrease in extracellular DA levels or an increase in D2 densities, but data from rodent studies have shown both effects resulting from exposure to enriched environments (e.g., Bowling et al. 1993; Hall et al. 1998; Zhu et al. 2004). Either mechanism could account for our findings in monkeys (see Nader and Czoty 2005 for additional discussion). The important point to highlight is the use of a within-subjects design to better understand how environmental variables affect behavior and brain function.

When D2 receptor availability is compared between the female monkeys in the Grant et al. (1998) study and the male monkeys in the Morgan et al. (2002) study, important similarities become apparent. In particular, the mean D2 DVR for dominant females and males was 3.00 ± 0.12 and 3.04 ± 0.23, respectively, and for the subordinate females and males was 2.41 ± 0.17 and 2.49 ± 0.10, respectively. These findings suggest no sex differences. It is important to note, however, that PET scans in the Grant et al. (1998) study were conducted while all monkeys were in the follicular phase of the menstrual cycle to control for possible menstrual cycle effects on brain dopamine systems (Becker et al. 2001; Di Paolo et al. 1986; for review see Becker 1999). Recent work in our laboratory confirmed that menstrual cycle phase can influence measures of D2 receptor availability (Czoty et al., in review). In contrast to the earlier studies (Grant et al. 1998; Morgan et al. 2002), these experiments used a primate microPET camera with a resolution of approximately 2 millimeters (mm) (compared to approximately 9 mm on PET cameras used in the earlier studies). As is evident in a representative monkey (Figure 2), the menstrual cycle clearly affected D2 receptor availability, with significantly lower levels observed in the follicular phase compared to the luteal phase. If we consider the earlier findings suggesting no apparent sex differences and consider that in the females in the Grant et al. (1998)
study DVRs would most likely be higher in the luteal phase, this would suggest that, depending on the phase of the menstrual cycle, we might in fact observe sex differences in D2 receptor availability.

The studies described in this section show that PET studies can contribute to a better understanding of how drugs, environment, and hormone levels alter brain function. This information will have significant implications for the development of animal models and treatment alternatives for many human diseases.

Other Imaging Modalities

As reviewed above, PET and SPECT studies in nonhuman primates have provided unique information about brain function that complements imaging studies in humans. Historically, nearly all brain imaging studies of addiction in human and nonhuman subjects utilized these methods. In the past decade, however, developments in imaging procedures based on MRI techniques have provided novel approaches to understanding the addicted brain. Compared to PET and SPECT, these techniques offer the advantage of increased spatial (1 mm) and temporal (seconds) resolution. Moreover, because MRI-based imaging does not require subjects’ exposure to radiation, it is safer than PET and SPECT and allows for individual subjects to be studied an unlimited number of times.

Encephalographic techniques provide more direct assessments of brain function, and with even greater temporal resolution (~1 millisecond). Although the application of these techniques to nonhuman primate models is in its infancy, it is likely that adaptation of these procedures will lead to significant advances in understanding the neurobiological factors that confer sensitivity to abused drugs and will aid in characterizing the long-term effects of drug use on brain function. We briefly review several of these techniques below.

Perfusion Techniques

Like PET, MRI has been used to examine cerebral blood flow (for review see Wintermark et al. 2005). There are two general methods for imaging blood flow that differ according to the approach to producing contrast (i.e., a differential intensity of signal based on tissue characteristics and imaging parameters). In one approach, called dynamic susceptibility contrast (DSC) MRI, contrast is provided by an exogenous paramagnetic agent administered as a bolus injection before imaging (e.g., Belliveau et al. 1990). This approach is similar to the use of $^{15}$O-labelled water in PET studies, but the signal in DSC MRI is based on the paramagnetic properties of the contrast agent rather than emitted radioactivity. In another technique, called arterial spin labelling (ASL), contrast is provided by labelling protons in arterial blood upstream of the brain region of interest (Williams et al. 1992). Both techniques offer clear advantages over PET and SPECT: DCS MRI does not require exposure to radiation, and ASL is completely noninvasive.

Because use of cocaine and amphetamines is associated with cardiovascular complications and an increased incidence of stroke in human addicts (e.g., Kaku and Lowenstein 1990; Lange and Hillis 2001), the ability to longitudinally study the effects of cocaine exposure on cerebral perfusion in nonhuman primates could provide information that will aid in treating cardio- and cerebrovascular complications associated with long-term cocaine use. Moreover, although studies have documented the acute vasoconstrictive effects of cocaine in recreational cocaine users (Kaufman et al. 1998a,b, 2001) and decreased perfusion in cortical and subcortical structures in abstinent abusers (Ernst et al. 2000), within-subject studies in monkeys could address whether these effects are preexisting traits in individuals vulnerable to the abuse-related effects of cocaine or are consequences of long-term cocaine exposure.

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**Figure 2** PET image of DA D2 receptors in a female monkey during the luteal phase (left panel) and the follicular phase (right panel) of the menstrual cycle. The original figure is in color and is available in the online posting of this article at www.ilarjournal.com.
Functional MRI (fMRI) and Pharmacological MRI (phMRI)

Among brain imaging techniques, functional MRI (fMRI) provides the highest spatial resolution for mapping brain function. The technique is based on neurovascular coupling, the local increase in blood flow that occurs to provide energy to regions of the brain in which activity has increased in response to a stimulus. As a result of this increased perfusion, the ratio of oxygenated to deoxygenated hemoglobin increases. Distortion of the magnetic field by deoxygenated hemoglobin can be detected with the use of appropriate imaging parameters (for review see Ogawa et al. 1990). The parameters used for fMRI result in a type of contrast that is dependent on the homogeneity of the magnetic field in each voxel. The intensity of signal in each voxel is modulated by blood flow, and thus is called blood oxygen level-dependent (BOLD) contrast. The BOLD signal is superimposed on an anatomical map to localize the response with excellent spatial resolution. On such a map, the pixel intensity represents the strength of correlation between the BOLD signal and the presentation of the stimulus, with different colors corresponding to positive and negative correlations.

fMRI has greatly advanced our understanding of brain reward circuitry and the neuropharmacological effects of cocaine in addicts (for review see Breiter and Rosen 1999; Volkow et al. 2004). In particular, fMRI studies in cocaine abusers have identified brain areas activated by cocaine or cocaine-associated environmental stimuli (e.g., Breiter et al. 1997; Goldstein et al. 2007; Martin-Soelch et al. 2001; Rislinger et al. 2005). A key issue that cannot be resolved in these human studies is whether differences observed between cocaine addicts and non-drug-using control subjects represent preexisting traits or the effects of drug exposure. Although fMRI studies in monkeys would be invaluable in assessing these possibilities, such studies have yet to directly address addiction. In fact, it is only in the past decade that researchers first used monkeys as subjects in fMRI studies investigating the visual system (Dubowitz et al. 1998; Stefanacci et al. 1998). An important feature of this work is the demonstration that imaging of awake monkeys is feasible (Logothetis et al. 1999; for review see Andersen et al. 2002).

More recent studies using fMRI to study brain activation by drugs rather than by visual stimuli have provided information more directly relevant to addiction. Whereas fMRI has proven invaluable in characterizing the brain’s response to exteroceptive stimuli, the BOLD contrast approach has been used to study effects of drugs that interact with brain dopamine, serotonin, opioid, glutamate, GABA, and cannabinoid systems in rodents (for review see Steward et al. 2005). This implementation of fMRI has been termed pharmacological MRI (phMRI). Research on addiction has shown that cocaine, amphetamine, heroin, and the cannabinoid CB1 receptor agonist HU210 produce BOLD responses in areas of the rodent brain relevant to the reinforcing effects of drugs, including the striatum, nucleus accumbens, and prefrontal cortex (Y. Chen et al. 1997; Marota et al. 2000; Shah et al. 2004; Xi et al. 2002).

Use of phMRI in nonhuman primates has been less frequent and only indirectly related to addiction research. phMRI studies in monkeys have been limited to characterization of alterations in the function of brain dopamine systems in relation to aging and in models of Parkinson’s disease (Jenkins et al. 2004; Q. Chen et al. 1999; Zhang et al. 2001, 2006). For example, in cynomolgus monkeys rendered parkinsonian by administration of the selective dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), amphetamine-induced increases in blood flow were reduced compared to unlesioned control subjects (Jenkins et al. 2004). Importantly, orderly relationships have been observed between the extent of the MPTP lesion as determined with PET or histological examination, the severity of resulting motor impairment, and reductions in effects of dopaminergic drugs on blood flow (Jenkins et al. 2004; Zhang et al. 2006). These results agree with most studies in rodents that demonstrate that the data obtained with phMRI correlate with measures of extracellular dopamine in addition to PET and behavioral data (Y. Chen et al. 1997, 1999; Nguyen et al. 2000).

The primary limitation of fMRI and phMRI, both in humans and in nonhuman primates, is the incomplete understanding of the relationship between neuronal activity and changes in blood flow. For example, although studies have shown that changes in the BOLD signal co-localize with changes in neural activity (e.g., Kim et al. 2004; Logothetis et al. 2001), interpretation of decreases in the functional response remains unclear. It is not mechanistically necessary that inhibition of activity in a brain area be accompanied by a decrease in the BOLD signal or vice versa. Moreover, interpretations of fMRI and phMRI data are limited by a reliance on relative rather than quantitative measures, the possibility that drugs can influence vascular function independent of neural activity, an incomplete understanding of age-related changes in perfusion, and a relatively low signal-to-noise ratio.

In addition to these inherent limitations, widespread application of fMRI and phMRI to addiction research in nonhuman primates faces several obstacles. Foremost is the necessity to eliminate any motion of the subject during imaging. As mentioned above, previous studies have demonstrated that training a monkey to remain perfectly still for an extended time is not impossible (e.g., Andersen et al. 2002; Howell et al. 2001), but it is extremely time- and labor-intensive, requiring several weeks to months of acclimating the monkey to the apparatus. Even if such training is successful, investigators must address the possibility that negative affective states such as stress or discomfort compromise the results. Nonetheless, such studies can be invaluable in characterizing the brain’s response to stimuli associated with cocaine. As an alternative to imaging awake monkeys, phMRI studies could be conducted while subjects are anesthetized. However, questions remain in such experiments...
about interactions between the drug of interest and anesthesia, as discussed above for PET imaging. These limitations are active areas of research in both human and nonhuman subjects (e.g., Keliris et al. 2007; Raemaekers et al. 2006; Shmuel et al. 2006; Sotero and Trujillo-Barreto 2007).

Diffusion Tensor Imaging (DTI)

A recently developed variant of MRI, diffusion tensor imaging (DTI\textsuperscript{1}), makes it possible to examine the integrity of axonal projections by measuring the direction of water diffusion in the brain (for reviews see Minati et al. 2007b; Mori and Zhang 2006; Taylor et al. 2004). In gray matter, water tends to diffuse relatively randomly in all directions (“isotropic diffusion”). In white matter, however, diffusion is restricted by axonal membranes and myelin sheaths. Because white matter is tightly packed into directionally oriented fiber bundles, water in a given area of white matter tends to diffuse along a vector parallel to the fiber tract (“anisotropic diffusion”) rather than randomly. Using appropriate MRI parameters, it is possible to generate gradients sensitive to diffusion along a certain vector that describes water movement in each voxel. From these data, eigenvectors are calculated for each voxel that describe the extent to which water flows along the fiber tract (\(\lambda_1\)) and perpendicular to the tract (\(\lambda_p\)). The tendency of water to flow along the fiber tract rather than perpendicular to it is determined by the amount of resistance to flow provided by the axonal membrane and myelin sheath (Beaulieu 2002). Typically, the principal eigenvector in each voxel is color coded on a map of the whole brain, in which different colors are assigned to different axes (i.e., anterior-posterior, dorsal-ventral, and left-right). Connecting adjacent voxels of similar directionality reveals the trajectories of fiber pathways (Basser et al. 2000). The main advantages of DTI compared to ex vivo tract-tracing methods are that DTI is less time consuming and that multiple experiments are possible in individual subjects, making longitudinal investigations possible.

Beyond its utility as an in vivo tract-tracing technique, DTI is useful for examining the integrity of axonal projections. The variable fractional anisotropy (FA) describes reductions in anisotropy that may be related to underlying pathology. Such reductions have been observed in several clinical populations compared to normal control subjects, including alcoholics and cocaine abusers (Lim et al. 2002; Moeller et al. 2005, 2007; Pfefferbaum et al. 2000). Other recent studies have determined that individual eigenvectors can provide more specific information about the nature of the underlying pathophysiology. Lower values of \(\lambda_1\) (i.e., less diffusion along the direction of the fiber tract) with little change in \(\lambda_p\) indicate a reduction in the number of axons in the tract. Conversely, demyelination and/or damage to axonal membranes results in decreased \(\lambda_p\) values with little effect on \(\lambda_1\) (Arfanakis et al. 2002; Song et al. 2002, 2005). This technique has obvious clinical use in diagnosing damage from a variety of sources. Using this approach, recent studies by Moeller and colleagues (2007) provided evidence of demyelination in cocaine users.

Although most studies using DTI have involved human subjects, DTI has been performed in nonhuman primates in tract-tracing studies (Parker et al. 2002) as well as in investigations of the effects of development and aging on brain connectivity (Kroenke et al. 2006; Makris et al. 2007). Application of DTI to addiction research in nonhuman primates could facilitate the investigation of questions impossible to address in humans about vulnerability to the abuse-related effects of drugs. A critical question about these deficits is whether differences in FA in drug and alcohol abusers are preexisting abnormalities that render these individuals vulnerable to addiction or rather are the result of drug use. Longitudinal studies in monkeys to examine white matter integrity before, during, and after chronic drug exposure would help answer this question. Moreover, the use of DTI could contribute to a better understanding of the nature of drug-induced decreases in anisotropy in human addicts. Importantly, DTI is more sensitive to detecting such decrements than other methods involving conventional MRI (Klingberg et al. 2000).

Considering the relative newness of DTI as an imaging technique, some limitations of the approach are not surprising (Taber et al. 2002; Taylor et al. 2004). These include a lack of standard methods of voxel placement for statistical analysis and an incomplete understanding of potential confounding variables. Limitations inherent to the technique include individual differences in the topography of fiber tracts, sensitivity to subject movement, a low signal-to-noise ratio, and disturbances in the magnetic field in areas where brain and bone interface. Increasing the voxel size in an attempt to increase signal decreases resolution and increases the likelihood that partial volume effects will obscure results. In other words, a large voxel is more likely to contain a mixture of fibers running in different directions, complicating the interpretation of the average eigenvalue for that voxel. Identifying imaging parameters that will minimize these limitations is an active area of research (e.g., Jones et al. 1999; Pipe et al. 2002). For example, more recently developed DTI methods have the potential to improve resolution of white matter directionality in voxels that contain curved, crossing, or branching fibers (e.g., Hosey et al. 2005; Minati et al. 2007a; Tuch 2004; for review see Pagani et al. 2007).

Magnetic Resonance Spectroscopy (MRS)

Whereas fMRI and phMRI provide information about vascular responses to stimulus-evoked and drug-induced increases in neural activity, magnetic resonance spectroscopy (MRS\textsuperscript{1}) provides information about levels of chemicals in discrete brain regions (for review see Minati et al. 2007). The technique takes advantage of the differential sensitivity of atomic nuclei in different molecular environments to an
applied magnetic field to provide relative or, in some cases, quantitative measures of the concentration of specific metabolites. Most MRS studies focus on the hydrogen nucleus due to its abundance. Different hydrogen-containing molecular entities generate specific resonance patterns that can be distinguished at a sufficiently high magnet strength but that are typically difficult to separate at commonly used magnet strengths (i.e., 1.5 tesla). Thus, the set of molecules that can be visualized with MRS is limited. However, several studies have used MRS to examine the pharmacological effects of abused drugs and to characterize the effects of long-term drug use on brain function (for review see Magalhaes 2005). Perhaps the most relevant neurochemicals that can be measured with MRS are the neurotransmitters GABA (for reviews see Chang et al. 2003; Rothman et al. 1993) and glycine (Prescot et al. 2006), which were found to be lower in cocaine and alcohol abusers (Behar et al. 1999; Ke et al. 2004). Other chemicals of interest that can be measured with MRS include N-acetyl-aspartate (NAA), choline, and creatine. NAA is a neuron-specific component of myelin synthesis (D’Adamo Jr. et al. 1968), and the choline level includes several phospholipids involved in the synthesis of cell membranes. Decreases in the concentration of these molecules may signify cell loss, decreases in metabolism, and/or demyelination (e.g., Birken and Oldendorf 1989). Abstinent cocaine users showed increased concentrations of total creatine and myoinositol compared to controls, suggesting gliosis in frontal and temporoparietal regions (Chang et al. 1997, 1999). In contrast, lower levels of phosphocreatine were reported in heroin-dependent subjects during initial treatment with methadone compared to normal control subjects (Silveri et al. 2004). The use of MRS for longitudinal monitoring of concentrations of these chemicals in monkeys with long-term exposure to stimulant drugs could provide information about changes that occur in the brain (including neurotoxic effects) during both long-term drug exposure and abstinence.

Electroencephalography (EEG) and Magnetoencephalography (MEG)

The MRI-based techniques described above have enabled the study of basal and drug-induced changes in brain activity with temporal and spatial resolution superior to those of PET and SPECT. Because these techniques measure alterations in the magnetic field based on changes in brain vasculature, however, they provide only an indirect measure of neuronal activity. In contrast to these approaches, electroencephalography (EEG) and magnetoencephalography (MEG) make it possible to obtain direct information about neuronal activity (for reviews see Hamalainen et al. 1993; Lopes da Silva 2004). In addition to directly measuring electromagnetic signals resulting from neuronal activity, these techniques provide temporal resolution in the millisecond range.

Neuronal communication is based on small intra- and extracellular currents. Synchronous activation of large groups of neurons produces currents large enough to be measured as a difference in potentials between electrodes placed at different positions on the scalp. EEG thus represents the most direct measurement of neuronal activity possible with noninvasive imaging techniques. The primary limitation of EEG is that electrical signals are distorted as they pass through brain tissue, skull, and skin, complicating localization of the point of origin of the EEG signals. Thus, spatial resolution is relatively poor (cm) and primarily determined by the location of electrodes on the scalp.

The currents produced by neuronal activity create a small magnetic field that can be detected using MEG. But, unlike the electrical signal measured with EEG, the magnetic field is not sensitive to distortion. Thus, localizing the source of activity is easier and more accurate with MEG, and excellent temporal resolution is maintained. Whereas EEG has been used in humans for decades to measure the effects of drugs—including drugs of abuse such as alcohol, cocaine, and heroin—on brain function (e.g., Franken et al. 2004; for reviews see Alper 1999; Little 1999; Saletu et al. 2002), MEG has been applied for this purpose only recently (for review see Kahkonen 2005a). A few published studies have used MEG to investigate the effects of alcohol on brain activity in human subjects (Kahkonen 2005b; Nikulin et al. 2005), and recent work has demonstrated the sensitivity of the MEG signal to drugs that act on monoamine systems (e.g., Kahkonen and Ahveninen 2002; Kahkonen et al. 2002; Pekkonen et al. 2002). Extension of these approaches to nonhuman primates will provide invaluable information about the specific involvement of drug effects in the long-term abnormalities observed in drug abusers.

Conclusions

The goal of this review was to briefly describe in vivo imaging techniques in use in nonhuman primates as well as those used to study human subjects that can be applied to nonhuman primate models in the future. In vivo imaging has many benefits that continue to expand our understanding of brain function. We have described imaging studies involving nonhuman primate models of drug abuse, but the methods are also effective for smaller animals and for questions related to any human disease. The greatest advantages of in vivo imaging techniques are the use of within-subjects designs and the ability to conduct longitudinal studies. We have also described some of the limitations of these imaging techniques, to be addressed with additional controlled studies in the future.

It is clear that in vivo imaging with animal models of human disease will not only provide the basis for understanding genetic predispositions and environmental modulation of many human diseases but also enhance the evaluation of novel treatment strategies. In this era of translational medicine, imaging techniques involving animal subjects will provide insights into the diagnosis, treatment, and prognosis of human disease.
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