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Title page

Nonhuman Primate PET Neuroimaging in Drug Abuse Research

L.L. Howell and K.S. Murnane

Leonard Lee Howell, Ph.D

Kevin Sean Murnane, Ph.D.

LLH, KSM Division of Neuroscience, Yerkes National Primate Research Center, Atlanta, GA USA

LLH Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA, USA

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Nonhuman Primate PET and Drug Abuse

Corresponding author:

Leonard L. Howell

Address:

Yerkes National Primate Research Center

Emory University

954 Gatewood Road

Atlanta, GA 30329

Ph.: (404) 727-7786

Fax: (404) 727-1266

Email: lhowell@emory.edu

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Nonstandard abbreviations

FECNT = 8-(2-fluoroethyl)-2-carbomethoxy-3-(4-chlorophenyl) nortropane; CFT = 2beta-carbomethoxy-3beta-(4-fluorophenyl)tropane; ZIENT = 2beta-carbomethoxy-3beta-[4'-((Z)-2-iodoethenyl)phenyl]nortropane; DASB = 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile; McN5652 = trans-1,2,3,5,6,10 beta-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]-isoquinolone); DTBZ = (+)-alpha-Dihydrotetrabenazine; SCH23390 = 7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol; Raclopride = (S)-(-)-3,5-dichloro-N-[(1-ethyl-2-pyrrolidinyl)]methyl-2-hydroxy-6-methoxybenzamide; Fallypride = (S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-fluoropropyl)-2,3-dimethoxybenzamide; MNPA = (R)-2-(11)CH(3)O-N-n-propylnorapomorphine; FCP = fluoroclebopride; FDG = fluorodeoxyglucose.

Abstract

Positron emission tomography (PET) neuroimaging in nonhuman primates has lead to significant advances in our current understanding of the neurobiology and treatment of stimulant addiction in humans. PET neuroimaging has defined the *in vivo* biodistribution and pharmacokinetics of abused drugs and related these findings to the time-course of behavioral effects associated with their addictive properties. With novel radiotracers and enhanced resolution, PET neuroimaging techniques have also characterized *in vivo* drug interactions with specific protein targets in brain, including neurotransmitter receptors and transporters. *In vivo* determinations of cerebral blood flow and metabolism have localized brain circuits implicated in the effects of abused drugs and drug-associated stimuli. Moreover, determinations of the predisposing factors to and long-term neurobiological consequences of chronic drug use, such as potential neurotoxicity, have lead to novel insights regarding the pathology and treatment of drug addiction. However, similar approaches clearly need to be extended to drug classes other than stimulants. Although dopaminergic systems have been extensively studied, other neurotransmitter systems known to play a critical role in the pharmacological effects of abused drugs have been largely ignored in nonhuman primate PET neuroimaging. Finally, the study of brain activation with PET neuroimaging has been mostly replaced in humans by functional magnetic resonance imaging (fMRI). Recently, there has been some success in implementing pharmacological fMRI in awake nonhuman primates. Nevertheless, the unique versatility of PET imaging will continue to complement the systems level strengths of fMRI, especially in the context of nonhuman primate drug abuse research.

Introduction

Non-invasive neuroimaging techniques have lead to significant advances in our current understanding of the neurobiology and treatment of drug addiction in humans. In positron emission tomography (PET) imaging, ligands of interest are radiolabeled with unstable atomic isotopes (see Phelps and Mazziotta, 1985; Senda et al., 2002; Fowler et al., 2007). Detector arrays and computer algorithms map the source and concentration of the radiotracer. Numerous radiotracers have been developed for use in PET neuroimaging that enable *in vivo* measurement of brain neurochemistry and physiology (Table 1). PET neuroimaging has defined the *in vivo* biodistribution and pharmacokinetics of abused drugs and related these findings to the time-course of behavioral effects associated with their addictive properties. With novel radiotracers and enhanced resolution, PET neuroimaging techniques have also characterized *in vivo* drug interactions with specific protein targets in brain, including neurotransmitter receptors and transporters. *In vivo* determinations of cerebral blood flow and metabolism have begun to localize brain circuits implicated in the effects of abused drugs and drug-associated environmental stimuli. Moreover, documentation of the long-term neurobiological consequences of chronic drug use and potential neurotoxicity has lead to novel insights regarding the pathology and treatment of drug addiction.

Parallel neuroimaging studies in nonhuman primates and human subjects provide a powerful translational approach that can link findings from humans and laboratory animals. In particular, nonhuman primate models allow for initially drug-naïve subjects in longitudinal designs to characterize within-subject changes in aspects of the neurobiology associated with chronic drug use. Moreover, experimental drugs under investigation can be evaluated in subjects

with well-documented drug histories. As with all animal models, enhanced experimental control is a noted advantage over the necessary restrictions imposed in human clinical research. Nonhuman primates also offer distinct advantages over other laboratory animal species. For example, their longevity is advantageous for many experimental designs. Compared to rodents, nonhuman primates are more similar to humans in the pharmacokinetics and metabolism of several drug classes including 3,4-methylenedioxymethamphetamine (MDMA) (Banks et al., 2007; Weerts et al., 2007). Furthermore, the brain distribution of PET radiotracers exhibits some heterogeneity even within primates, suggesting greater differences may be encountered when comparisons are drawn across orders (Yokoyama et al., 2010). Lastly, nonhuman primates exhibit complex social behaviors that provide unique opportunities for examining the influence of social variables on the abuse-related effects of drugs (Morgan et al., 2002; Nader and Czoty, 2005; Nader et al., 2008). With few exceptions, drug abuse research utilizing PET neuroimaging in nonhuman primates has focused on cocaine and related stimulants. Accordingly, the current review will focus on abused stimulants.

Drug Mechanism of Action

Biodistribution

PET neuroimaging in nonhuman primates has been critical to understanding the mechanism of action of cocaine and related stimulants. An early study focused on the distribution of cocaine binding in the brain of anesthetized baboons using [^{11}C]-labeled cocaine (Fowler et al., 1989). Cocaine binding was heterogeneous but showed some selectivity for

dopamine transporter (DAT) rich striatal regions. Striatal cocaine binding was inhibited by pretreatments with pharmacological doses of cocaine and DAT inhibitors but not by norepinephrine transporter (NET) or serotonin transporter (SERT) inhibitors. Direct comparisons in human subjects showed a similar distribution of binding with the highest concentration in the striatum. A subsequent study documented significant overlap in the distributions of binding of [^{11}C]- labeled cocaine and methylphenidate (Volkow et al., 1995). Importantly, a direct relationship was established between self-reports of "high" induced by cocaine and the time-course of striatal uptake (Volkow et al., 1997). More recently, the brain pharmacokinetics of methamphetamine were compared to cocaine in anesthetized baboons using [^{11}C]-labeled d-methamphetamine and (-) cocaine (Fowler et al., 2007). The results indicated that the slower clearance of methamphetamine compared to cocaine likely contributed to its longer lasting stimulant effects. Finally, the reinforcing effects of several cocaine analogs were compared to the time-course of uptake of the [^{11}C]-labeled drugs in the putamen of awake rhesus monkeys (Kimmel et al., 2008). The cocaine analogs were reliably self-administered but rates of responding were lower than those maintained by cocaine. Importantly, there was a clear trend towards an inverse relationship between the time to peak uptake of [^{11}C]-labeled drugs in putamen and the peak number of intravenous (i.v.) infusions received, such that the faster-onset drugs produced greater levels of responding relative to the slower-onset drugs. There was also a close correspondence between the time-course of drug uptake in brain and drug-induced increases in extracellular dopamine in caudate (Czoty et al., 2002; Ginsburg et al., 2005; Kimmel et al., 2007; Kimmel et al., 2008). These studies clearly show that PET biodistribution measures inform our understanding of drug mechanism of action and concomitant behavioral effects, such

as reinforcing and subjective effects. However, as these studies exclusively examined the brain biodistribution of stimulants, it remains to be determined if these techniques will prove to be as useful when applied to other drug classes.

Drug Occupancy

PET neuroimaging in nonhuman primates has documented the relationship between drug occupancy at monoamine transporters and the behavioral effects of stimulants. For example, PET imaging in rhesus monkeys using the [^{18}F]-labeled FECNT, a DAT selective radioligand, showed that FECNT labels a cocaine-sensitive binding site, and high levels of DAT occupancy are associated with behaviorally-active cocaine doses (Votaw et al., 2002). More recently, the reinforcing effects of local anesthetics that bind to the DAT *in vitro* were evaluated for DAT occupancy *in vivo* in rhesus monkeys (Wilcox et al., 2005). Doses of dimethocaine that maintained maximum response rates produced DAT occupancies between 66-82%. These values are highly concordant with results from human PET imaging studies, which found that DAT occupancies were between 60-77% for cocaine doses that subjects reported as rewarding (Volkow et al., 1997). They are also concordant with PET imaging data in rhesus monkeys, which revealed that cocaine DAT occupancies between 65-76% maintain peak response rates (Wilcox et al., 2002). Unlike dimethocaine, procaine was ineffective in maintaining self-administration and resulted in DAT occupancies between 10-41% (Wilcox et al., 2005). However, irrespective of the drug, *in vivo* microdialysis showed that reinforcing effects and DAT occupancy were closely related to drug-induced increases in extracellular dopamine. These

studies illustrate the power of PET imaging to unmask drug mechanism of action, particularly as it relates to monoamine transporters, and they highlight the utility of the translational nature of PET imaging in nonhuman primates. Consistent with these findings, it has been shown recently that DAT occupancy by methylphenidate is highly concordant between rhesus monkeys and humans when blood levels of the drug are matched (Wilcox et al., 2008).

PET imaging has also been used to study protein occupancy by other stimulants. The interoceptive and reinforcing effects of the substituted phenethylamine MDMA share some similarity to other stimulants (Fantegrossi et al., 2002; Murnane et al., 2009) but the role of the DAT in the behavioral effects of MDMA is not well documented, especially in primates. Accordingly, a recent study assessed the role of the DAT in the behavioral effects of racemic MDMA in nonhuman primates (Fantegrossi et al., 2009). A dose of MDMA that suppressed operant behavior had negligible effects on extracellular dopamine in the caudate, and the percent DAT occupancy of MDMA was marginal. This work was subsequently corroborated by findings that higher doses of MDMA were required to elicit significant release of dopamine (Murnane et al., 2010). Collectively, these results indicate that the rate suppressant effects of MDMA are not mediated by the DAT and that MDMA has lower potency at the DAT compared to other stimulants, such as cocaine or amphetamine. Similar to MDMA, the role of the DAT in the behavioral effects of the wake promoting drug modafinil is not well documented. Importantly, a number of clinical studies suggest that modafinil may improve clinical outcomes for treatment of cocaine dependence by reducing self reports of craving and cocaine-induced euphoria (Dackis et al., 2003; Dackis et al., 2005; Hart et al., 2008; Anderson et al., 2009) through a possible DAT-mediated mechanism (Volkow et al., 2009; Zolkowska et al., 2009). To this end, a recent study

in rhesus monkeys demonstrated that the *in vivo* effects of modafinil at the DAT are similar to other stimulants, such as cocaine (Andersen et al., 2010). Modafinil-induced nocturnal locomotor-stimulant effects and reinstated previously extinguished cocaine self-administration. An effective dose of modafinil resulted in approximately 60% DAT occupancy in the striatum and significantly increased extracellular dopamine levels, comparable to effects observed following cocaine doses that reliably maintain self-administration (Ito et al., 2002; Votaw et al., 2002; Wilcox et al., 2002; Wilcox et al., 2005). The results obtained provide important information about the mechanism of action of modafinil and show low potency DAT-related effects in nonhuman primates. Although these studies collectively demonstrate the power of PET imaging to characterize the transporter-related effects of stimulants, the relationship between presynaptic transporter effects and postsynaptic dopamine receptor effects requires further elucidation.

Neurotransmitter Release

Competition between radiolabeled ligands and endogenous neurotransmitters provides an effective means of evaluating drug-induced changes in extracellular neurotransmitter concentrations *in vivo* (see Laruelle, 2000). Similar to other PET measures, findings in nonhuman primates and human subjects exhibit considerable overlap (Table 2). PET neuroimaging with [^{18}F]-labeled fluoroclebopride (FCP) as a reversible D2 receptor ligand characterized stimulant-induced dopamine release in rhesus monkeys (Mach et al., 1997). Intravenous administration of cocaine, amphetamine, methylphenidate and methamphetamine each increased rates of FCP washout from the basal ganglia, consistent with the capacity of each drug to elevate extracellular dopamine. [^{11}C]-labeled raclopride studies in baboons (Dewey et al.,

1992; Villemagne et al., 1998; Volkow et al., 1999) and [^{18}F]-labeled fallypride studies in rhesus monkeys (Mukherjee et al., 1997) documented that these effects can be demonstrated with several radioligands and in several primate species. More recent studies in baboons and rhesus monkeys have begun to document the usefulness of drug-induced displacement of [^{18}F]-labeled fallypride to characterize dopamine release in extrastriatal brain regions (Mukherjee et al., 1997; Slifstein et al., 2004). Taken together, these results validate the use of PET imaging as an *in vivo* measure of neurotransmitter release in nonhuman primates and provide a solid foundation for human studies (Martinez et al., 2007, Volkow et al., 2008). However, most studies have been limited to dopamine displacement of D2 binding in the striatum. In apparent contrast to the effects of dopamine releasers, the serotonin releaser fenfluramine significantly increased extracellular serotonin in rhesus monkeys without displacing the [^{18}F]-labeled $5HT_{1A}$ receptor ligand MPPF (Udo de Haes et al., 2006). Moreover, dopamine depletion did not affect the binding potential of the [^{11}C]-labeled D2 receptor ligand FLB 457 in human cortex (Frankle et al., 2010). The relative affinity of the radioligand and the endogenous neurotransmitter, protein density in specific brain regions, and direct interactions between the drug and its metabolites with the protein target are all important considerations that may influence the outcome and interpretation of *in vivo* displacement studies.

Recently, PET imaging has been used in nonhuman primates to examine the receptor pharmacology influencing dopamine release. In one study, pretreatment with the mGluR1 receptor antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) attenuated dopamine release by methamphetamine, as measured by [^{11}C]-labeled MNPA (Tokunaga et al., 2009). Similarly, the mGluR2 agonist LY354740 potentiated amphetamine-elicited dopamine release, as measured by

[^{11}C]-labeled raclopride (van Berckel et al., 2006). Similar to biodistribution studies, displacement of radiotracers by drug-induced increases in neurotransmitter levels can be used to study the time-course of drug action (Narendran et al., 2007). In addition, the importance of the intrinsic efficacy of PET radioligands has recently become recognized. For example, the D2 receptor agonist radioligand MNPA is more sensitive than the D2 antagonist radioligand raclopride to amphetamine-elicited increases in dopamine levels (Seneca et al., 2006). This is consistent with prior *in vitro* competition binding work showing that agonists have a higher apparent affinity for receptors labeled with agonist radioligands than antagonist radioligands (Sleight et al., 1996). Moving forward, these pharmacodynamic and pharmacokinetic studies are likely to yield new insights into mechanisms that modulate the neurochemical and behavioral effects of drugs of abuse.

Cerebral Blood Flow and Metabolism

The noninvasive measurement of cerebral blood flow with PET neuroimaging and [^{15}O] water provides a useful means to characterize acute drug-induced changes in brain activity. Early studies in human cocaine abusers using SPECT imaging largely showed regional decreases in cerebral blood flow following acute administration of cocaine (Pearlson et al, 1993; Wallace et al, 1996; Johnson et al, 1998). Functional changes in cerebral blood flow using PET imaging have also been determined in awake, drug-naïve rhesus monkeys following acute i.v. cocaine administration (Howell et al., 2001; Howell et al., 2002). In contrast to the deactivations found in humans with a history of cocaine abuse, brain activation maps normalized to global flow showed prominent cocaine-induced activation of prefrontal cortex, especially dorsolaterally. These differences in the direction of the change in blood flow induced by cocaine may have been due to

differences in the methodology implemented, the dose of cocaine tested, the drug history of the subject, or other factors. Nevertheless, importantly, the selective SERT inhibitor alaproclate attenuated the brain activational effects of cocaine, cocaine-induced increases in striatal dopamine, and self-administration of cocaine in rhesus monkeys(Czoty et al., 2002; Howell et al., 2002). Hence, there was close concordance between *in vivo* measures of behavior, neurochemistry and functional imaging. A more recent study was the first to use PET imaging with [^{15}O] water to document acute cocaine-induced changes in brain activity during cocaine self-administration in nonhuman primates (Howell et al., 2010). The area of major activation included anterior cingulate cortex, a region associated with the extended limbic system. Furthermore, drug-associated stimuli elicited increases in regional cerebral blood flow in the dorsomedial prefrontal cortex, indicating robust cortical activation. Consistent with these findings, a recent study in cocaine-addicted humans showed that a dose of methylphenidate that increased dopamine levels, as indexed by displacement of [^{11}C]-labeled raclopride binding, only elicited drug "craving" when presented in combination with drug-associated stimuli (Volkow et al., 2008). In another study in nonhuman primates, functional changes in glucose metabolism were characterized in rhesus monkeys with [^{18}F]-labeled flurodeoxyglucose (FDG) following acute administration of cocaine (Henry et al., 2010). Similar to the results obtained using [^{15}O] water to map cerebral blood flow, metabolic mapping demonstrated acute cocaine-induced activation of extended limbic regions in cocaine-naïve rhesus monkeys. Collectively, these studies enhance our understanding of the neurobiological effects of drugs of abuse. However, although extensive PET imaging work has examined dopaminergic mechanisms (Figure 1) of

drugs of abuse in nonhuman primates, a relative paucity of work regarding other mechanisms of action, such as serotonergic effects, has been published.

Biomarkers

Neurobiological Changes

A major advantage of PET imaging is the ability to employ longitudinal designs that involve repeated measures over extended periods of time. This approach has been used effectively in nonhuman primates to characterize enduring changes in brain chemistry associated with chronic drug exposure (Table 3). For example, PET imaging studies conducted in socially housed cynomolgus monkeys characterized the effects of chronic cocaine exposure in dominant and subordinate subjects. Although dominant monkeys initially exhibited higher D2 receptor availability and lower rates of cocaine self-administration (Morgan et al., 2002), chronic exposure to self-administered cocaine resulted in reductions in D2 receptor availability to levels comparable to subordinate monkeys (Czoty et al., 2004). A subsequent study examined D2 receptor availability during extended cocaine abstinence (Nader et al., 2006). In subjects with short-term exposure, D2 receptor availability returned to pre-drug levels within three weeks. In subjects with long-term exposure, some showed complete recovery within three months, whereas others did not recover after one year of abstinence. However, the rate of recovery did not correlate with total drug intake. Nevertheless, individual differences in D2 receptor availability recovery rates have also been observed following drug-induced increases by D2 receptor antagonists (Czoty et al., 2005). An early study of cocaine self-administration effects on DAT

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levels largely showed increases in DAT levels, but in a dose, brain region, and exposure duration dependent fashion (Letchworth et al., 2001). A study of DAT availability using [^{18}F]-labeled FCT examined the effects of cocaine self-administration in rhesus monkeys under contingencies that resulted in low drug intake (Czoty et al., 2007). Self-administration of a low cocaine dose over nine weeks did not significantly affect DAT availability in any brain region. After more prolonged histories of cocaine self-administration, significant increases in striatal SERT were observed using [^{11}C]-labeled DASB (Banks et al., 2008). In other work, a non-contingent dosing regimen of morphine decreased DAT availability in rhesus monkeys (Xiao et al., 2006). Collectively, these studies demonstrate substantial but yet to be fully elucidated plasticity of the dopaminergic system in response to exposure to drugs of abuse.

Evaluations of drug-induced changes in protein binding *in vivo* are complemented by a recent study that documented cocaine-induced changes in brain metabolic activity as a function of cocaine self-administration history (Henry et al., 2010). Experimentally naive rhesus monkeys were given increasing access to cocaine self-administration. PET neuroimaging with [^{18}F]-labeled FDG was used to measure acute cocaine-induced changes in brain metabolism in the cocaine-naïve state, and following limited- and extended-access conditions. In the cocaine-naïve state, cocaine-induced increases in brain metabolism were restricted to the anterior cingulate and medial prefrontal cortex. Increased cocaine exposure from limited through extended access recruited cocaine-induced metabolic effects in additional frontal cortical areas and within the striatum. In apparent contrast, tolerance to cocaine- and amphetamine-induced synaptic release of dopamine in the striatum was observed in these same animals under both access conditions (Kirkland Henry et al., 2009). Interestingly, blunting of dopamine release has also been recorded

in cocaine dependent humans, using [^{11}C]-labeled raclopride (Martinez et al., 2007). Furthermore, this blunting of dopamine release was associated with whether the subject would choose cocaine over money. Accordingly, further combined study of the relationship between drug self-administration and both tolerance to the dopaminergic effects of cocaine and its recruitment of cortical activation may be highly relevant toward efforts to develop treatments for cocaine addiction. Indeed, a treatment that reverses these effects may have significant clinical value.

There has been significant interest in the potential neurotoxic effects of amphetamine derivatives, such as methamphetamine and MDMA. Under a variety of conditions, MDMA has selective and enduring effects on markers of brain serotonin systems, which some investigators interpret as neurotoxicity. However, early studies were limited by biochemical and histological analyses that required between-subject comparisons. An early PET imaging study in a baboon characterized the effects of MDMA on *in vivo* SERT availability using [^{11}C]-labeled McN5652 (Scheffel et al., 1998). Following treatment with MDMA twice daily for four consecutive days, PET scans showed reductions in SERT availability in all brain regions analyzed at 13–40 days post-drug treatment but regional differences in its apparent recovery at 9 and 13 months. Similar results have been reported for methamphetamine-induced reductions in DAT availability in baboons (Villemagne et al., 1998) and rhesus monkeys (Hashimoto et al., 2007). However, other studies provided more ambiguous results (Melega et al., 2008), including small and transient changes in D1 receptor availability using [^{11}C]-labeled SCH23390 (Hashimoto et al., 2007). Furthermore, behavioral decrements resulting from neurochemical changes induced by exposure to amphetamine derivatives have been much more difficult to establish (Winsauer et al., 2002;

Saadat et al., 2006). It is critical to note that studies reporting neurotoxic effects of amphetamine derivatives have relied on non-contingent drug administration and have typically administered large and/or repeated doses. In one of the first studies to characterize the neurochemical effects of self-administered MDMA in nonhuman primates, rhesus monkeys self-administered MDMA for approximately 18 months. PET neuroimaging with [^{11}C]-labeled DTBZ was used to quantify vesicular monoamine transporter (VMAT) availability following at least two months of drug abstinence (Fantegrossi et al., 2004). The reinforcing effects of MDMA were selectively attenuated by chronic MDMA self-administration but there was no significant change in VMAT binding potential and no significant changes in serotonin or dopamine levels in postmortem brains. A more recent study found a similar lack of significant SERT availability changes following MDMA self-administration in rhesus monkeys using [^{11}C]-labeled DASB (Banks et al., 2008). Hence, non-contingent drug administration has yielded neurochemical changes in the absence of behavioral correlates whereas drug self-administration has yield behavioral alterations in the absence of any significant neurochemical correlates. As such, given the important public health implications of drug-induced neurotoxicity, further study is clearly warranted. In this regard, PET imaging in rhesus monkeys has shown that pre- or post-exposure treatment with the antibiotic minocycline prevents methamphetamine-elicited reductions in DAT availability (Hashimoto et al., 2007). Such an approach may be highly beneficial in the prevention or treatment of any neurotoxic effects of amphetamine derivatives.

Vulnerability Factors

It has become well accepted that behavior, brain chemistry and neuronal function can be readily influenced by pharmacological challenges and by environmental conditions. Differences

in the dominance rank among socially-housed nonhuman primates have been associated with differential levels of dopamine D2 receptors as measured with [^{18}F]-labeled FCP (see Nader and Czoty, 2005). Social housing of male cynomolgus monkeys increased the availability of D2 receptors in dominant animals without producing any changes in subordinate group members, and these changes appeared to exert significant effects on cocaine self-administration (Morgan et al., 2002). Cocaine reliably functioned as a reinforcer in subordinate subjects but failed to maintain self-administration in dominant monkeys. Similarly, subordinate animals were more sensitive to the reinforcing effects of cocaine evaluated with a choice procedure, such that they would choose a lower dose of cocaine over food compared to dominant animals (Czoty et al., 2005). Additionally, the protective effects associated with high D2 receptor density in dominant animals can be attenuated with prolonged exposure to cocaine (Czoty et al., 2004). Hence, a trait variable that is associated with low vulnerability to stimulant abuse may become less important with continued exposure to stimulants. Further, the observation that female cynomolgus monkeys show significant changes in D2 binding potential associated with menstrual cycle phase may have direct relevance to understanding the neurobiological basis of human sex differences in sensitivity to stimulants (Czoty et al., 2009). The success of these studies suggests that the identification of additional vulnerability factors other than D2 levels would be of great benefit to drug abuse research.

Medications Development

Despite extensive efforts directed toward the development of medications to treat cocaine abuse, no effective pharmacotherapy is currently in clinical use. Given the important role of DAT in the addictive properties of cocaine, the development of compounds that target the DAT

represents a reasonable approach for the pharmacological treatment of cocaine abuse. A series of studies was conducted in nonhuman primates that evaluated the effectiveness of DAT inhibitors in reducing cocaine self-administration. PET neuroimaging quantified DAT occupancy at behaviorally-relevant doses, characterized the time-course of drug uptake in brain, and documented drug-induced changes in cerebral blood flow as a model of brain activation. Selective DAT inhibitors were effective in reducing cocaine self-administration but only at high (>70%) levels of DAT occupancy. For example, effective doses of the DAT-selective inhibitor RTI-113, which dose-dependently reduced cocaine-maintained responding, produced DAT occupancies between 72-84% (Wilcox et al., 2002). Similar results were observed with other DAT-selective inhibitors, including the phenyltropane RTI-177 and the phenylpiperazine GBR 12909 (Lindsey et al., 2004). Importantly, selective SERT inhibitors were also effective in reducing cocaine self-administration and blocked cocaine-induced brain activation and increases in extracellular dopamine (Czoty et al., 2002; Howell et al., 2002). Similarly, a mixed-action inhibitor of DAT and SERT, RTI-112, significantly reduced cocaine self-administration by rhesus monkeys at doses producing levels of DAT occupancy below the limit of detection (Lindsey et al., 2004). Furthermore, co-administrations of the selective SERT inhibitors fluoxetine or citalopram and the selective DAT inhibitor RTI-336 produced more robust reductions in cocaine self-administration than RTI-336 alone, even at comparable levels of DAT occupancy by RTI-336 (Howell et al., 2007). Collectively, it appears that serotonergic effects enhance suppression of cocaine self-administration by DAT inhibitors, indicating that duel DAT/SERT inhibitors warrant consideration as viable medications for cocaine addiction.

Translational Value of Nonhuman Primate Neuroimaging

With few exceptions, functional neuroimaging studies in nonhuman primates have utilized Old World macaques and baboons. These animals offer important advantages over other species of laboratory animals for the study of drug abuse. For example, the nonhuman primate prefrontal cortex is anatomically and functionally homologous to human prefrontal cortex, which is a brain region that, as previously discussed, has marked relevance for drug abuse. Nonhuman primates have a sophisticated behavioral repertoire, which allows for the study of complex schedules of reinforcement and cognitive processes important for studying the etiology, maintenance, and consequences of drug abuse. Nonhuman primate social behavior, including the development of social hierarchies, has demonstrated relevance for studying drug abuse. Drug metabolic and pharmacokinetics effects in nonhuman primates are arguably more similar to human drug metabolism and pharmacokinetics than those of other laboratory animal species. The life span of nonhuman primates is long and exhibits similarities to humans, such as delayed maturation and prolonged adolescence, that may be relevant for studying drug abuse, and are well suited for long-term longitudinal studies using the relatively non-invasive techniques of neuroimaging. Of these examples, perhaps the area that is most germane to neuroimaging and of greatest translational value is the homology of the nonhuman primate prefrontal cortex to human prefrontal cortex because of the relevance of drug effects on prefrontal cortical function to human drug addiction. Functional brain imaging in humans has begun to define the neural circuitry underlying the acute pharmacological effects of cocaine, conditioned responses to cocaine-cues and the experience of drug craving in humans. Activation of the anterior cingulate and dorsolateral prefrontal cortex has been observed in response to acute administration of

cocaine and related stimulants (Breiter et al. 1997; Volkow et al. 1999; Kufahl et al. 2005) and cocaine-related environmental cues (Maas et al. 1998; Childress et al. 1999; Kilts et al. 2001; Wexler et al. 2001; Maas et al. 1998; Grant et al. 1996). The anterior cingulate, part of the extended limbic system, is anatomically linked to the prefrontal cortex and nucleus accumbens, and serves diverse functions including the integration of mood and cognition (Vogt et al. 1992; Devinsky et al. 1995). The dorsolateral and dorsomedial prefrontal cortices are activated during the performance of a variety of cognitive tasks that require working memory or goal-directed behavior (Fuster 1997). Hence, it is apparent that the effects of cocaine and associated cues extend beyond the limbic system to engage brain areas underlying complex cognitive processes. Importantly, the same neuroanatomical regions as reported in humans subjects are activated during cocaine self-administration and extinction in rhesus monkeys, establishing strong validity for the nonhuman primate model employed (Howell et al, 2002; 2010). Elevations in rates of glucose utilization in the same brain areas following cocaine self-administration in rhesus monkeys have also been reported (Porrino et al. 2002; Henry et al, 2010). Obviously, self-reports of drug craving cannot be obtained in animal studies. However, the distinct pattern of brain activation observed in nonhuman primates may provide a novel functional measure to assess interventions designed to attenuate cue-induced changes in brain activity. These translational neuroimaging studies of drug dependence have been complemented by behavioral measures associated with drug use and relapse following periods of drug abstinence. Collectively, neuroimaging studies in nonhuman primates have identified neuronal targets to effectively reduce cocaine use and have characterized underling neurochemical mechanisms associated with potential therapeutic effects. The identification of neural circuits underlying the direct

pharmacological and conditioned-stimulus effects of cocaine may be highly relevant toward efforts to develop pharmacological treatments for drug addiction.

Limitations and Future Directions

The use of PET neuroimaging in nonhuman primates has advanced our understanding of the neurobiological basis of stimulant addiction, providing an effective translational approach for medications development and treatment of stimulant abuse in humans. However, similar approaches clearly need to be extended to other drug classes with high abuse liability. Although the importance of dopamine in drug addiction is well recognized, other neurotransmitter systems known to play a critical role in the pharmacological effects of abused drugs have been largely ignored in nonhuman primate PET neuroimaging. There has been some progress in the development of techniques to study serotonergic and glutamatergic systems, and a comprehensive understanding of the neurobiology underlying drug addiction will likely depend on the continued development of such novel approaches. For example, the identification of vulnerability factors other than dopamine D2 receptor availability would be highly beneficial. Also, *in vivo* PET measures of neurotransmitter release in nonhuman primates have been limited to dopamine displacement of D2 receptor binding in the striatum. However, it remains to be determined whether neurotransmitters other than dopamine reliably displace PET ligand binding at alternative targets in nonhuman primates, and it will be important to validate these displacement studies with direct measures of neurotransmitter levels derived from *in vivo* microdialysis. Finally, the study of brain activation by PET imaging with [^{15}O] water and FDG

has been mostly replaced in humans by functional magnetic resonance imaging (fMRI) because of the higher temporal and spatial resolution and lack of radiation exposure with this imaging modality. Recently, there has been some success in implementing pharmacological fMRI in awake nonhuman primates (Jenkins et al., 2004; Brevard et al., 2006; Murnane and Howell, 2010; Figure 2). However, there are significant challenges associated with the conduct of fMRI imaging in awake nonhuman primates as it is inherently more sensitive to subject motion than PET imaging and requires restraint equipment built entirely from non-ferrous materials. Despite these challenges, fMRI should prove to be highly effective in characterizing drug-induced changes in brain activity at a systems level. Nevertheless, the unique versatility and specificity of PET imaging will continue to complement the systems level strengths of fMRI, especially in the context of nonhuman primate drug abuse research.

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Footnotes

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Figure legends

Figure 1

Elements of the dopamine neurovascular unit that have been previously imaged using PET imaging in nonhuman primate drug abuse research. As can be seen, numerous radiotracers have been developed and validated for studying a broad range of dopaminergic elements. The key for the elements is inset.

Figure 2

Brain activation elicited by cocaine and MDMA in a single nonhuman primate imaged using fMRI. Each drug was administered at 0.3mg/kg i.v. and the subject was fully conscious during the scan. Images are presented as coronal sections of the prefrontal cortex. As noted, efforts are underway to extend PET brain activation findings using this imaging modality. As can be seen, fMRI finely localizes a different systems level activation elicited by each drug of abuse. The statistical value of the change in activation is inset and expressed in units of the t statistic value.

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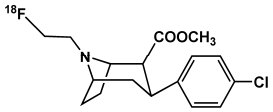
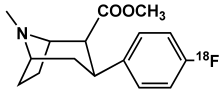
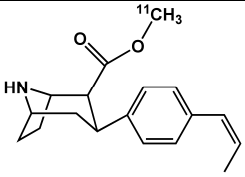
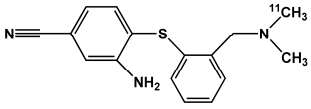
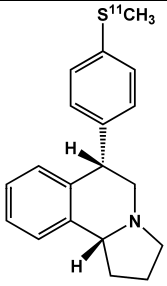
Table 1

Radiotracers used in previously published nonhuman primate drug abuse research. Isotopes in brackets indicate the isotope most often used to radiolabel the tracer.

Name / Acronym	Protein target	Structure	Action	Common uses	Selected studies
[^{18}F]-FECNT	Dopamine transporter		Inhibitor	Occupancy Availability	Lindsey et al., 2004 Howell et al., 2007 Fantegrossi et al., 2009 Andersen et al., 2010
[^{18}F]-CFT	Dopamine transporter		Inhibitor	Occupancy Availability	Hashimoto et al., 2007
[^{18}F]-ZIENT	Serotonin transporter		Inhibitor	Occupancy Availability	Lindsey et al., 2004
[^{11}C]-DASB	Serotonin transporter		Inhibitor	Occupancy Availability	Banks et al., 2008
[^{11}C]-McN5652	Serotonin transporter		Inhibitor	Occupancy Availability	Scheffel et al., 1998

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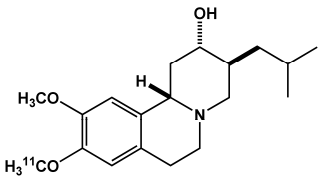
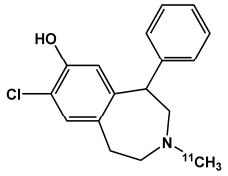
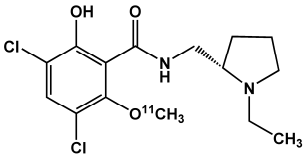
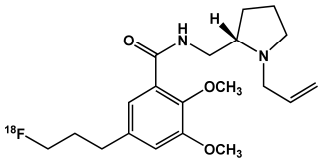
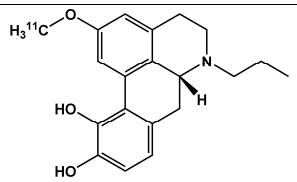
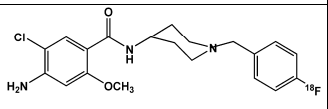
[^{11}C]-DTBZ	Vesicular monoamine transporter		Inhibitor	Occupancy Availability	Fantegrossi et al., 2004
[^{11}C]-SCH 23390	Dopamine D1 receptors		Antagonist	Occupancy Availability	Hashimoto et al., 2007
[^{11}C]-Raclopride	Dopamine D2 receptors		Antagonist	Occupancy Availability	Dewey et al., 1992 Villemagne et al., 1999 Volkow et al., 1999 van Berkel et al., 2006 Seneca et al., 2006
[^{18}F]-Fallypride	Dopamine D2 receptors		Antagonist	Occupancy Availability	Mukherjee et al., 1997 Slifstein et al., 2004 Mukherjee et al., 2005
[^{11}C]-MNPA	Dopamine D2 receptors		Agonist	Occupancy Availability	Seneca et al., 2006 Tokunaga et al., 2009
[^{18}F]-FCP	Dopamine D2		Reversible	Occupancy	Mach et al., 1997 Czoty et al., 2004

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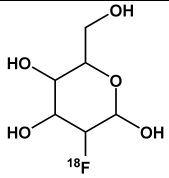
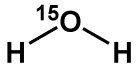
	receptors		ligand	Availability	Nader and Czoty, 2005
[^{18}F]-FDG	Mitochondria		Substrate	Metabolism	Henry et al., 2010
[^{15}O]-Water	None		Substrate	Blood flow	Howell et al., 2001 Howell et al., 2002 Howell, 2010
Labeled Drugs (e.g. cocaine or MDMA)	Various	Various	Various	Occupancy Availability Drug distribution	Fowler et al., 1989 Fowler et al., 2007 Kimmel et al., 2008

FECNT = 8-(2-fluoroethyl)-2-carbomethoxy-3-(4-chlorophenyl) nortropane; CFT = 2beta-carbomethoxy-3beta-(4-fluorophenyl)tropane; ZIENT = 2beta-carbomethoxy-3beta-[4'-((Z)-2-iodoethenyl)phenyl]nortropane; DASB = 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile; McN5652 = trans-1,2,3,5,6,10 beta-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]-isoquinolone); DTBZ = (+)-alpha-Dihydrotetrabenazine; SCH23390 = 7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol; Raclopride = (S)-(-)-3,5-dichloro-N-[(1-ethyl-2-pyrrolidinyl)]methyl-2-hydroxy-6-methoxybenzamide; Fallypride = (S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-fluoropropyl)-2,3-dimethoxybenzamide; MNPA = (R)-2-(11)CH(3)O-N-n-propylnorapomorphine; FCP = fluoroclebopride; FDG = fluorodeoxyglucose.

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Table 2

Comparison of the acute effects of drugs of abuse in nonhuman primate or human subjects as measured by PET or SPECT neuroimaging

Effect	Drug	Nonhuman primates	Humans
Cerebral blood flow	Cocaine	↑ [1,2,3]	↓ [4,5,6]
Cerebral metabolism	Cocaine	↑ [7]	↓ [8]
DAT binding	Cocaine, Modafinil	↑ [9,10,11,12]	↑ [9]
Correlation between brain kinetics and reinforcing or interoceptive effects	Cocaine, Cocaine analogs	Yes [13]	Yes [14]
Correlation between protein occupancy and reinforcing or interoceptive effects	Cocaine, Modafinil, Local anesthetics	Yes [10,11,12,13]	Yes [14]
Displacement of D2 receptor selective ligands	Cocaine, Amphetamine, Methylphenidate, Methamphetamine	↑ [17,18,19,20,21,22,23,24]	↑ [25,26]

1: Howell et al. 2001; 2: Howell et al. 2002, 3: Howell et al. 2010, 4: Pearlson et al. 1993, 5: Wallace et al. 1996, 6: Johnson et al. 1998, 7: Henry et al., 2010, 8: London et al. 1990, 9: Fowler et al., 1989, 10: Wilcox et al., 2002, 11: Votaw et al., 2002, 12: Wilcox et al., 2005, 13: Andersen et al., 2010, 14: Volkow et al., 1997, 15: Kimmel et al., 2008, 16: Volkow et al., 1997,17: Dewey et al., 1992, 18: Mach et al., 1997, 19: Mukherjee et al., 1997, 20: Villemagne et al., 1998, 21: Volkow et al., 1999, 22: Slifstein et al., 2004, 23: van Berckel et al., 2006, 24: Tokunaga et al., 2009, 25: Martinez et al., 2007, 26: Volkow et al., 2008

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Table 3: Long-term consequences of exposure to drugs of abuse in nonhuman primates measured using PET neuroimaging

Effect	Drug	Radiotracer
Recruitment of brain activation	Cocaine	[^{18}F]-FDG [1]
No change in DAT availability	Cocaine	[^{18}F]- FCT [2]
Decreased DAT availability	Methamphetamine	[^{11}C]-WIN-35,428 [3]
		[^{18}F]-CFT [4]
No change in D1 dopamine receptor availability	Methamphetamine	[^{11}C]-SCH23390 [4]
Decreased D2 receptor availability	Cocaine	[^{18}F]-FCP [5,6,7]
Increased SERT availability	Cocaine	[^{11}C]-DASB [8]
Decreased SERT availability	MDMA	[^{11}C]-McN5652 [9]
No change in SERT availability	MDMA	[^{11}C]-DASB [8]

No change in VMAT availability	MDMA	[^{11}C]-DTBZ [10]

1: Henry et al., 2010, 2: Czoty et al., 2007, 3: Villemagne et al., 1998, 4: Hashimoto et al., 2007, 5: Czoty et al., 2004, 6: Czoty et al., 2007, 7: Nader et al., 2006, 8: Banks et al., 2008, 9: Scheffel et al., 1998, 10: Fantegrossi et al., 2004

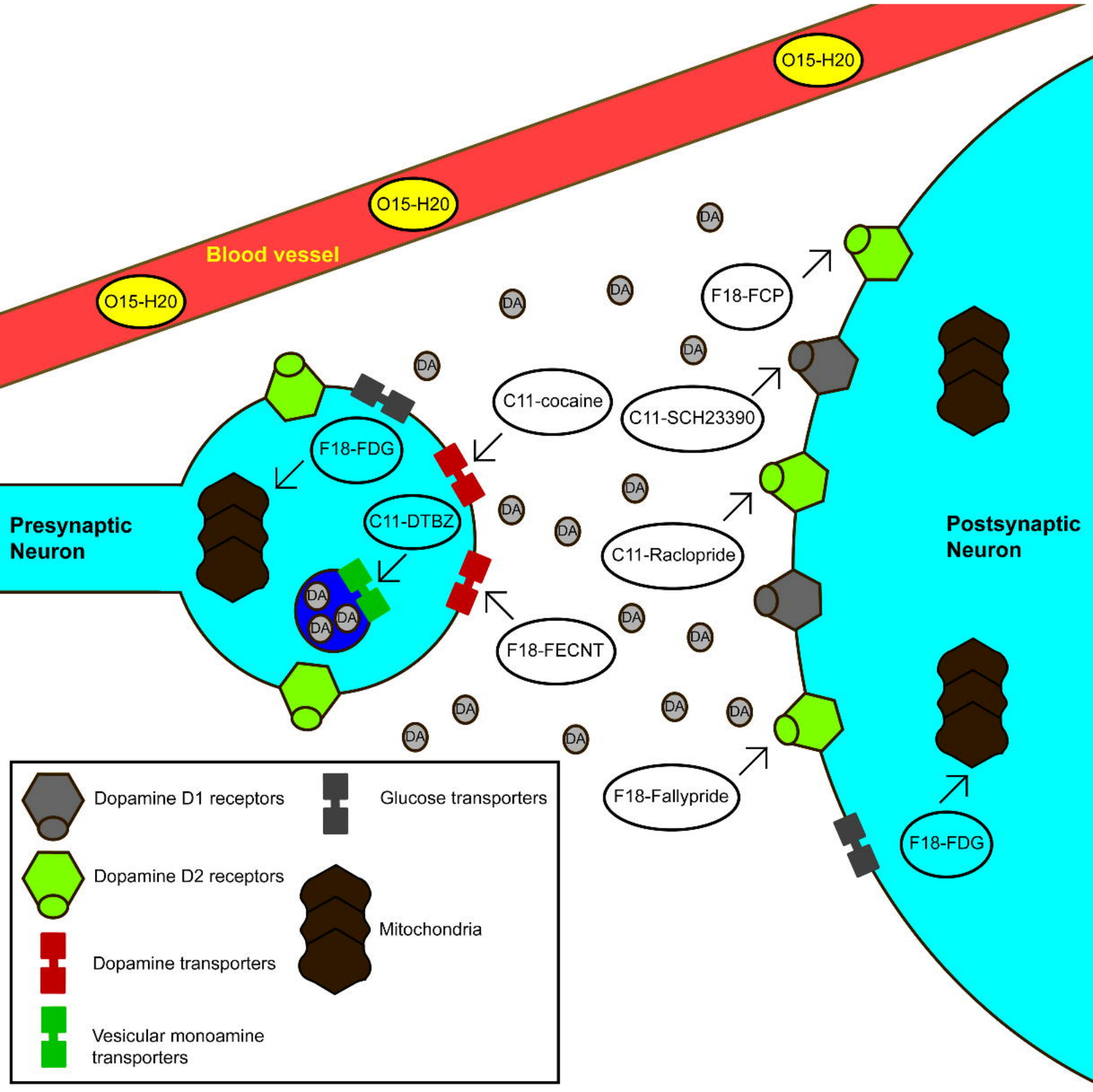
O15-H20
O15-H20
Blood vessel
O15-H20
DA
F18-FCP
C11-cocaine
C11-SCH23390
F18-FDG
C11-DTBZ
Presynaptic Neuron
Postsynaptic Neuron
C11-Raclopride
F18-FECNT
F18-Fallypride
F18-FDG
Dopamine D1 receptors
Glucose transporters
Dopamine D2 receptors
Mitochondria
Dopamine transporters
Vesicular monoamine transporters

Figure 1

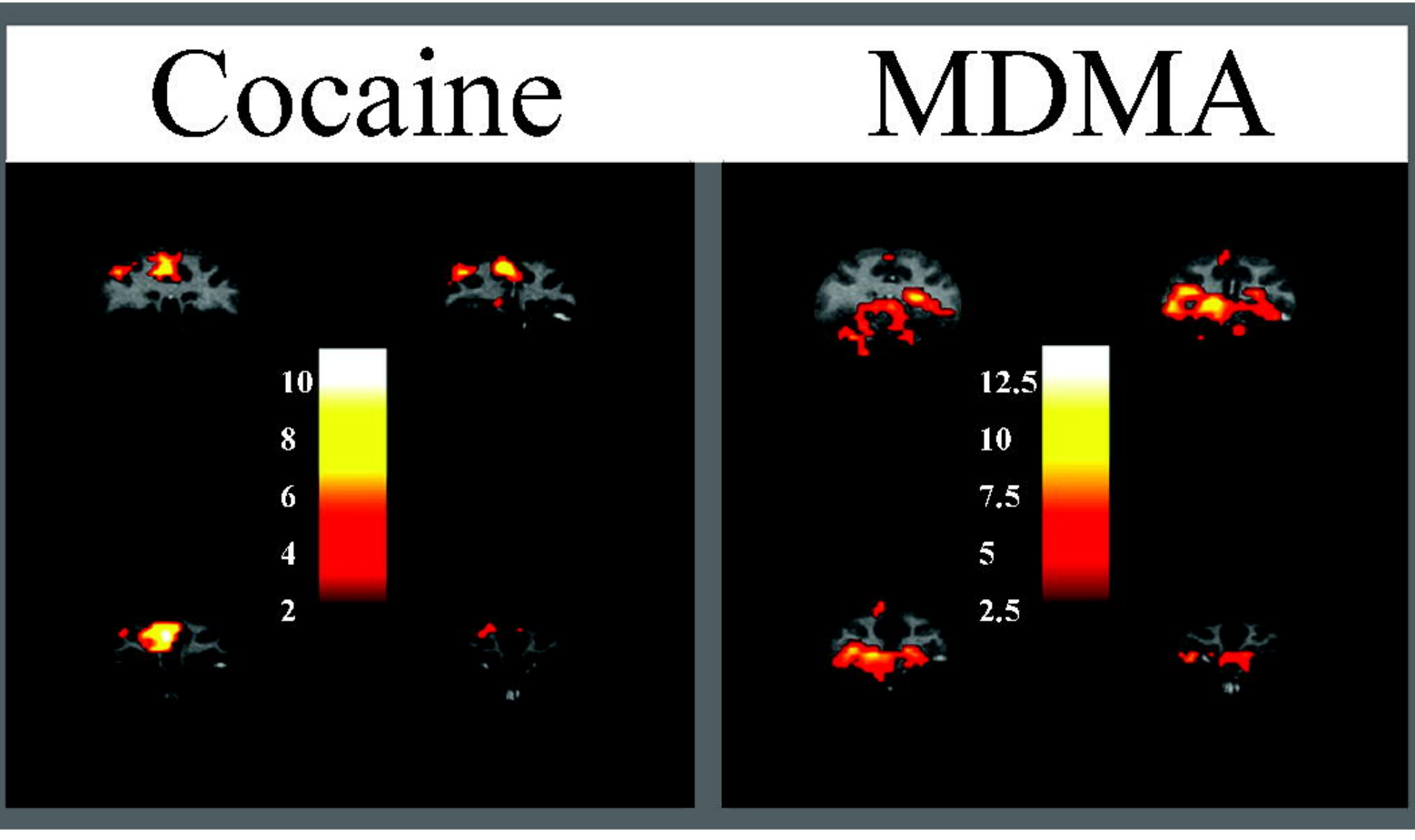

Figure 2