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Determining Dopamine D1 Receptor Changes in Cocaine Abusers Using Positron Emission Tomography and SCH 23390

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Abstract

An underlying mechanism of cocaine is its ability to bind to the dopamine transporter thus preventing dopamine (DA) reuptake, and leading to an increase of endogenous DA in the synapse. Excessive synaptic DA may lead to neuroadaptions of the DA receptors. *In vitro* animal studies have demonstrated a cocaine-induced down-regulation of D1 receptors in limbic regions of the brain. PET studies in rats have also noted a decrease in D1 receptor numbers in the striatum. In humans, D1 receptors have not been examined using either *in vitro* or PET techniques. The present investigation was conducted to determine if there were changes in D1 receptor density in cocaine abusers. Eight cocaine-addicted were examined within their first week of abstinence using PET and the D1-receptor radioligand SCH 23390. The binding potentials (BP) of the nucleus accumbens, putamen and caudate nucleus were obtained and compared to eleven control-group subjects. The results indicate no group differences in BP between the two groups. There was no correlation found between amount and duration of cocaine use and BP. There was a significant negative correlation between age and BP. The results suggest that, contrary to findings obtained in animal research, there is no evidence of D1 receptor changes in chronic cocaine abusers compared to controls.

Résumé

Le mécanisme sous-jacent de la cocaïne est sa capacité de se lier au transporteur de la dopamine, empêchant ainsi la réabsorption de la dopamine (DA), et causant un excès de DA endogène dans la jonction synaptique. La DA synaptique excessive peut mener à des adaptations neurologiques des récepteurs de la DA. Des expériences faites en laboratoire sur des animaux ont démontré une régulation à la baisse induite par la cocaïne des récepteurs D1 dans les zones limbiques du cerveau. Des études T.E.P. chez les rats ont aussi permis de noter une réduction du nombre de récepteurs D1 dans le striatum. Chez les humains, les récepteurs D1 n'ont pas été étudiés au moyen d'expériences en laboratoire, ni au moyen des techniques T.E.P. L'enquête en cours a été effectuée afin de déterminer s'il y avait des changements dans la densité du récepteur D1 chez les consommateurs réguliers de cocaïne. On a examiné huit cocaïnomanes durant leur première semaine d'abstinence en utilisant le T.E.P. et le radioligand SCH 23390 du récepteur D1. On a obtenu les possibilités de lien (PL) des noyaux accumbens, du putamen caudé et du noyau intraventriculaire du corps strié que l'on a comparées à onze sujets d'un groupe témoin. Les résultats indiquent qu'il n'existe pas de différence de PL entre les deux groupes. On n'a trouvé aucune corrélation entre la quantité et la durée de consommation de cocaïne et les PL. On a constaté une corrélation négative importante entre l'âge et les PL. Les résultats indiquent que, contrairement aux constatations obtenues en recherche animale, il n'y a aucun changement de récepteur de D1 évident chez les cocaïnomanes chroniques par rapport aux sujets contrôles.

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Introduction

Cocaine is an alkaloid derived from the leaves of the coca plant that has local anaesthetic and vasoconstrictor properties that has also been widely abused as a mental stimulant. The vasoconstriction and anaesthetic effects lead to serious cardiovascular and other medical complications, while its stimulant properties lead to its compulsive abuse (Bolla et al., 1998).

The leaves have been chewed for centuries in South America to reduce fatigue and hunger. In 1859, cocaine was isolated from coca leaves and it was used in medications to treat a wide variety of aliments, from digestive to psychological disorders. Within a few decades, it became evident that abuse and addiction were problems with this substance. The Harrison Act in 1914 reduced the availability of cocaine in the United States, while the 1910 Narcotics Control Act reduced its availability in Canada. These acts lead to a decline in the use of cocaine for the next 50 years. However, increased use began again in the 1960's and by the 1980's cocaine use had once more become a problem. Abuse worsened further with the advent of administration methods that permitted faster absorption of the drug, such as smoking and injecting (Benowitz, 1993).

Substance dependence is a chronically relapsing disorder that is characterised by three major elements: 1) compulsion to seek and take the drug, 2) loss of control in limiting intake, and 3) emergence of a negative emotional state when access to the drug is prevented (Koob and Le Moal, 1997). Cocaine dependence is associated with major medical, neurological, and neuropsychiatric complications (Bolla et al., 1998). Effective pharmacological methods of treatment for cocaine dependence have not been developed. It is hoped that the present research

will contribute to an understanding of cocaine abuse and aid in the development of pharmacotherapies.

The Dopamine System and Cocaine Addiction

Neuropharmacological studies have established an important role for the dopaminergic system in the acute reinforcing effects of cocaine (Koob et al., 1998). On a pharmacological level cocaine is a non-selective drug that interacts at a variety of distinct sites. It binds with high affinity to the transporter sites for the neurotransmitters dopamine (DA), serotonin (5-HT) and noradrenaline (NA) thereby inhibiting the reuptake of these amines into the pre-synaptic neurons. Cocaine also binds with moderate affinity to sodium channels, and has a lower affinity for opioid, muscarinic, cholinergic, adrenergic receptors, and calcium channels (Smith et al., 1999). DA is found throughout the brain, but mesocorticolimbic DA has been implicated in the reinforcing actions of abused drugs (Gong et al., 1999; Koob et al., 1994; Koob and LeMoal, 1997; Koob et al., 1998; Pulvirenti and Koob, 1994; Self, 1998; Xu et al., 2000). This area consists of DA-containing cells that originate in the ventral tegmental area (VTA) of the mid-brain. The cell bodies in the VTA send their axons to various structures including the nucleus accumbens (NAc), olfactory tubercle, frontal cortex and amygdala (White and Kalivas, 1998; Kiyatkin, 1994).

Manipulation of the DA system has been shown to alter cocaine-related behaviours. For example, neurotoxin-induced lesions of dopaminergic terminals in the NAc and cell bodies in the VTA have been shown to reduce cocaine self-administration in rats (Roberts and Koob,

1982; Zito et al., 1985). In addition, cocaine-related locomotor activity was blocked by lesions of the NAc (Kelly and Iversen, 1976), as well as by microinjections of the DA antagonist haloperidol into the NAc (Le Moal and Simon, 1991).

More recently Koob (1998) carefully examined the effects of DA receptor antagonists on intravenous cocaine self-administration in rats. Rats were trained to self-administer cocaine for three hours per day and showed a stable and regular drug intake over each daily session. The rats appeared to regulate the amount of drug self-administered. If the dose was lowered by 0.75 mg/kg per injection from that received during the training session, the rats increased the number of self-administered infusions. When low doses of DA-receptor antagonists were injected intravenously there was an increase in self-administration (Koob, 1998). This suggested that partial blockade of the DA receptors produces a partial blockade of the reinforcing actions of cocaine.

When cocaine is administered it concentrates in brain areas that are richest in DA. Using PET and [^{11}C] cocaine Fowler et al. (1989) found the highest concentration of cocaine in the striatum of humans and primates. In post-mortem studies with primates Kaufman et al. (1991) sought to identify specifically where cocaine binds in the brain. They used the cocaine analogue [^{3}H] CFT and performed quantitative autoradiography. High densities of [^{3}H] CFT binding in the caudate nucleus (CN), Putamen (PU), and the NAc were found. Intermediate levels of binding were noted in the amygdala and very low levels of binding were noted in the cerebellum. Telang et al. (1999) used PET and [^{11}C] cocaine to map the specific regions in the human striatum where cocaine binds. The largest amount of labelled cocaine was found in the PU, NAc,

and CN. Moderate binding was noted in the thalamus and amygdala and low-level binding was noted in the cerebellum. The above findings correspond to the known distribution dopamine in the brain.

The underlying mechanism of cocaine is its ability to block the DA transporter (DAT) (Telang et al., 1999). Using PET and [^{11}C] cocaine it has been shown that 60-80% occupancy of the DAT was required for induction of euphoria (Volkow et al., 1997). However, it is possible that DAT blockade is not solely responsible for cocaine's effects. Giros et al. (1996) developed a strain of DAT knockout mice. In these mice cocaine administration failed to alter baseline extra-cellular DA levels and it failed to induce enhanced locomotor activity and stereotypy. However, DAT knockout mice did self-administer cocaine (Rocha et al., 1998). These findings strongly suggested that other neurotransmitter systems, such as 5-HT, might be involved. However, DAT knockout mice are known to have compensatory changes in the pre- and post-synaptic receptors that are involved in DA neurotransmission (Jones et al., 1998).

Binding of cocaine to the DAT prevents DA reuptake leading to an excess of endogenous DA in the synapse (White and Kalivas, 1998). High synaptic DA levels lead to an increase in DA neurotransmission (Xu et al., 2000) and this increase is thought to contribute to the subjective and reinforcing effects of cocaine. Euphoria, reduced fatigue, psychomotor stimulation and improved mental clarity are among the effects caused by cocaine that are thought to lead to abuse (Smith et al., 1999). Brain microdialysis experiments permit direct measurement of changes in synaptic levels of neurotransmitters in conscious animals. A dose-dependent increase in extra-cellular NAc DA concentration has been reported after experimenter- and self-

administered cocaine (Di Chiara and Imperato, 1988; Kalivas and Duffy, 1990; and Petit and Justice, 1989). Cocaine also increased DA concentration in the striatum, VTA and pre-frontal cortex (Kalivas et al., 1993).

DA receptors are present both pre- and post-synaptically, as well as on the DA cell body itself. On the post-synaptic side, receptors function in cell-to-cell communication and on the pre-synaptic neuron they modulate the release and synthesis of DA. Autoreceptors exert feedback to the neuron itself in order to regulate DA synthesis, DA release and neuronal firing. Five DA receptor subtypes (D_1 - D_5) have been cloned and sequenced. D_1 and D_5 receptors are termed 'D_1-like' while D_2, D_3 and D_4 are grouped together and termed 'D_2-like'. The D1-like and D2-like receptors are distinguishable by their structural homology, opposite modulation of adenylate cyclase activity and differential localisation within the brain (Self, 1998). The highest concentration of D1 and D2 receptors occurs in the striatum (Volkow et al., 1996). Both families of DA receptors have been suggested to contribute differently to the reinforcing properties of cocaine (Self et al., 1996; Spealman et al., 1999). For the purposes of this paper, the focus will be on the role of DA D1-like receptors in cocaine addiction.

The influential role of DA D1 receptors in the effects of cocaine was illustrated in studies of genetically altered D1 receptor knockout mice (Xu et al., 1994). Acute doses of cocaine were ineffective in stimulating locomotor activity in these knockout mice, whereas wild-type mice exhibited a dose-related increase in locomotor activity. Xu et al. (2000) sought to further clarify the role that dopamine D1 receptors play in the behavioural effects of cocaine. They compared D1 receptor knockout mice to wild-type mice and examined responses to repeated cocaine

administration. Both types of mice were injected with cocaine or saline twice daily for seven consecutive days and their behavioural responses (i.e. locomotion, rearing and grooming) were noted. The D1 mutant mice had significantly lower locomotor responses (including rearing and grooming) compared to the wild-type mice. In addition, the D1 knockout mice had similar locomotor responses to both saline and cocaine. The authors concluded that D1 receptors play an essential role in mediating cocaine-induced behavioural changes in mice. The importance of D1 receptors in mediating locomotor responses may be indicative of the enabling role of these receptors. D1 receptor stimulation appears to be necessary for the electrophysiological and behavioural manifestations of the D2-class receptor stimulation (Waddington and Daly, 1993; White and Hu, 1993).

The Role of Dopamine D1-like Receptors in Cocaine Addiction as Ascertained by D1 Antagonists

Several studies have demonstrated that D1 antagonists attenuate the reinforcing effects of cocaine. Koob et al. (1987) found that rats increased their rate of self-administration when they were injected with the D1 antagonist SCH 23390 prior to cocaine administration. The increased lever pressing was thought to be indicative of a reduction in the reinforcing properties of cocaine. Similarly, the D1 antagonists SCH 39166 and SCH 23390 caused the cocaine dose-response curve to shift to the right in monkeys (Bergman et al., 1990; Katz et al., 1999). Studies that used intracranial microinjection of SCH 23390 have shown that the NAc (Maldonado et al., 1993) and sub-regions of the NAc and the amygdala (Caine et al., 1993) may be particularly sensitive to D1 antagonist blockade of the reinforcing effects of cocaine.

Katz et al. (1999) used mice to study the psychomotor stimulant and monkeys to study the subjective effects of several compounds that act at D1 receptors. The effects of the ligands were examined alone and in combination with cocaine. Locomotor activity in the mice was measured as an indicator of psychomotor stimulation, while in the monkeys the discriminative-stimulus effect and response rates were considered representative of the subjective effects. Antagonist compounds that had a higher D1 receptor affinity were more potent at decreasing motor activity in mice and response rates in monkeys. The D1 antagonists SCH 23390, SCH 39166 and A-69024 dose-dependently shifted the cocaine dose-response curve for locomotor activity and response rates to the right. The same compounds shifted the discriminative-stimulus effects of cocaine to the right. Overall, the results indicated that D1 receptors contribute to the behavioural effects of cocaine.

A recent human study by Romach et al. (1999) examined the effect of the selective D1/D5 antagonist ecopipam (SCH 39166) on the subjective responses to intravenous cocaine. Each test session began with a double blind oral ecoppipam dose of 10 mg, 25 mg or 100mg given two hours prior to intravenous administration of 30 mg of cocaine. Visual analogue scales (where 0 = not at all and 100 = the most ever) were scored by the subjects who rated feelings of "high," "anxious," "confused," "sedated," "good drug effect," "bad drug effect," and "desire to take cocaine." Ratings were obtained at -30, -15, 5, 15, 60, and 90 minutes relative to the cocaine administration. Subjective responses to the cocaine were attenuated in direct proportion to the dose of the antagonist.

Studies have suggested that D1 receptors are important in relapse to cocaine-seeking behaviour. The extinction-reinstatement paradigm is often used to assess relapse in animals. In this paradigm, animals are trained to intravenously self-administer cocaine (e.g. pressing on drug-paired lever) and the behaviour is later extinguished when saline is substituted for cocaine. Subsequently, priming doses of cocaine (i.e. non-contingent drug infusion) and/or receptor specific ligands are administered, and the effect of these pharmacological manipulations on relapse is then measured by noting a reinstatement to non-reinforced bar pressing activity.

The blockade of D1-like receptors by ecopipam (SCH 39166) dose-dependently attenuated the priming effect of cocaine in monkeys (Spealman et al., 1999). This effect is also seen with D1 agonists as discussed below. The similar effects of D1 antagonists and agonists on priming may indicate that there is a critical range of D1 receptor activity that enables reinstatement of cocaine-seeking behaviour. If this is the case D1 receptors may play a crucial modulatory role in cocaine priming and they may constitute viable targets for the development of medications to control relapse (Spealman et al., 1999).

The Role of Dopamine D1-like Receptors in Cocaine Addiction as Ascertained by D1 Agonists

Animal studies have indicated that D1 agonists are self-administered suggesting D1 receptor involvement in the reinforcing effects of drugs of abuse. Self and Stein (1992) found that rats self-administered the D1 agonists SKF 82958 and SKF 77434. Weed et al. (1993) trained monkeys to self-administer cocaine, and then substituted the D1 agonist SKF 81297 or saline for the cocaine once cocaine-responding levels were stable. SKF 81297 maintained responding above saline-responding levels for all monkeys. The D1 antagonist SCH 39166 was

then administered and the monkeys decreased their SKF 81297 responding rates in a dose-dependent manner.

Weed and Woolverton (1995) sought to determine differences in self-administration rates of three high-efficacy D1 agonists versus three low-efficacy D1 agonists. Monkeys were trained to lever press for cocaine and periodically D1 agonists were substituted for the cocaine. All monkeys self-administered the high-efficacy D1 agonists at rates that indicated that these compounds functioned as positive reinforcers. The low efficacy D1 agonists were not self-administered.

Self et al. (1996) conducted a series of extinction-reinstatement experiments to assess the effects of D1-like agonist SKF 82958 on reinstatement to cocaine-seeking behaviour. The D1-like agonists did not reinstate non-reinforced responding on a cocaine-paired lever. Self and Nestler (1998) demonstrated that cocaine was able to induce reinstatement of responding in previously cocaine dependent animals. However, when the animals were pre-treated with a D1-agonist the ability of cocaine to induce responding at the drug-paired lever was attenuated. This demonstrated that D1-like agonists are unlikely to elicit relapse. It also suggests that D1-like agonists may have some utility in the treatment of cocaine abuse.

Haney et al. (1999) worked with human subjects who were not interested in seeking treatment for their cocaine problem. The authors pre-treated the subjects with intravenous doses of the potent and selective D1 agonist ABT-431 and then provided the opportunity for cocaine self-administration. The effects of ABT-431 on cocaine craving, subjective-effects ratings and cocaine self-administration were ascertained. ABT-431 caused a significant dose-dependent

decrease in the subjective effects of cocaine and decreased cocaine craving. However, ABT-431 had no effect on cocaine self-administration. The authors suggest that this D1 agonist warrants further investigations as a potential treatment for cocaine abuse despite the fact that it did not alter self-administration. They hypothesised that attenuating cocaine craving and its subjective effects may influence cocaine use in individuals who are seeking treatment for their cocaine dependence. They also suggest that a medication would have to decrease the subjective effects markedly before cocaine self-administration is affected, and that higher doses of ABT-431 may be appropriate. However, D1 receptor agonists have been shown to be reinforcing in rats and non-human primates (Caine and Koob, 1994; Self and Stein, 1992; Weed et al., 1993; Weed and Wolverton, 1995) so their utility in the treatment of cocaine dependence may be questionable. Although D1 receptor agonists have been shown to maintain IV self-administration in animals, the available evidence suggests that the abuse potential of some of these drugs is less than that of cocaine (Caine et al., 1999; Grech et al., 1996; Self and Stein, 1992; Weed et al., 1997). In addition, reinforcing drugs do have clinical potential as they can increase treatment compliance. Medications currently used to treat alcohol (benzodiazepines) and heroin (methadone) addiction are themselves addictive but they have been shown to be useful in the treatment of these disorders (Haney et al., 1999). Self (1998) suggests that if a D1 agonist was given in a slow-onset, long-acting formulation the drug may block craving without producing reinforcing effects.

Neuroadaptive Changes in Post-Synaptic Dopamine D1 Receptors due to Prolonged Cocaine Exposure

At its core, addiction involves a biological process; in particular, the effects over time of repeated exposure to an agent (drug) on a biological substrate (brain) (Nestler and Aghajanian, 1997). Drug exposure causes adaptations in individual neurons thus altering the functioning of the neural circuits in which these neurons operate (Nestler and Aghajanian, 1997). The repeated use of cocaine can result in enduring alterations in the behavioural effects of acute drug administration (Bartlett et al., 1997). The molecular and cellular basis for these alterations may reside in the neuroadaptations arising in the same neural elements that mediate the acute reinforcing actions of cocaine (Koob et al., 1998).

The use of cocaine is associated with acute increases in DA levels in the synaptic cleft, and over-stimulation of post-synaptic DA receptors. Used regularly, cocaine causes chronic over-stimulation of the receptors. Down-regulation of the post-synaptic receptors has been proposed as the neuroadaptive mechanism used to deal with the excessive DA levels (Bolla et al., 1998).

Dopamine D1 Receptor Findings with Chronic Cocaine Use

To date there are no research studies examining D1 receptor changes in cocaine-addicted humans. However, *in vitro* techniques have demonstrated a down-regulation of D1 receptors in the limbic regions of animals that self-administered or were administered cocaine. Kleven et al. (1990) sought to ascertain if chronic administration of cocaine produced long-lasting alterations in dopamine-receptor binding. They treated rats with single daily injections of cocaine (0, 10 or 20 mg/kg) for 15 consecutive days and sacrificed them either 20 minutes or two weeks after the

last injection. They found that the number of D1 binding sites in the striatum was decreased 20 minutes and two weeks after the last injection. In the NAc there was a decrease at the 20-minute point. Laurier et al. (1994) noted a transient decrease in the number and agonist sensitivity of dopamine D1 receptors in the limbic region in rats that were trained to self-administer cocaine. One week following the last infusion of cocaine, D1 receptor numbers had returned to normal levels in the limbic region but remained low in the forebrain. Moore et al. (1998) examined the effects of chronic self-administration of cocaine on D1-like receptors in rhesus monkeys. The authors compared the brains of three rhesus monkeys that had been self-administering 1.35 mg/kg of cocaine per day for 18-22 months to cocaine-naive monkeys. They used SCH 23390 and in vitro quantitative autoradiography to quantify binding densities in the striatum. They found that the binding density was reduced in regions of the striatum especially in the NAc. The shell of the NAc showed the largest difference with significantly lower D1 binding also detected in adjacent regions of the CN and the PU. The authors concluded that cocaine modulated the density of D1 receptors in specific portions of the primate striatum and that such changes might underlie drug dependence and craving.

De Montis et al. (1998) examined rats that were trained to self-administer cocaine intravenously. The rats were placed in triads such that one rat was trained to self-administer cocaine and the other two received either cocaine or saline on the same schedule as the rat self-administering cocaine (i.e. yoked saline or yoked cocaine). After 30 days of stable responding, the rats were sacrificed to ascertain D1 receptor density using SCH 23390. The brain areas that were examined included the olfactory tubercle, prefrontal cortex, NAc, striatum, and

hippocampus. Animals receiving cocaine (both self-administered and yoked) showed a down-regulation of D1 receptor density in the areas examined.

Positron Emission Tomography (PET)

PET was developed as a pharmacological tool to measure receptor concentrations in living organisms (Stoessl and Ruth, 1998). It is a tracer technique that makes it possible to detect accurately and non-invasively *in vivo* concentrations of positron-labelled compounds. The technique involves labelling various pharmacological agents with unstable radionuclides that decay by positron emission. Several D1 receptor antagonists have been labelled with positron emitters these include [^{11}C] SCH 23390, [^{11}C] SCH 39166, [^{11}C] NNC687, [^{11}C] NNC 756 and [^{11}C] NNC ^{11}2 (Volkow et al., 1996). A problem with most D1 receptor radioligands is that they also have an affinity for 5-HT_2 receptors. However, there is high binding of these D1 radioligands in the striatum corresponding to the area of highest D1 receptor concentration (Volkow et al., 1996).

The most commonly used D1 receptor radiotracer is [^{11}C] SCH 23390. [^{11}C] SCH 23390 has been shown to be a reliable and reproducible ligand for the study of D1 receptor binding by PET (Chan et al., 1998) and it has also been shown to be unaffected by changes in endogenous levels of DA (Thibaut et al., 1996; Abi-Dargham et al., 1999; Chou et al., 1999).

PET Findings for D_1 Receptors in Cocaine Studies

Tsukada et al. (1996) sought to develop an animal model of human cocaine consumption patterns. Cocaine abusers often self-administer cocaine repeatedly over a short period of time

(Pulvirenti and Koob, 1994). The model developed by Tsukada et al. (1996) included three doses of cocaine administered one hour apart followed by no administration for 22 hours. Rats were assigned to one of six experimental groups where saline or cocaine was administered for 2, 7 or 14 days. The rats were injected with the D1 radioligand SCH 23390 and scanned to measure D1 receptor binding. The scans showed that the 7 and 14-day cocaine groups had a significant decrease in SCH 23390 binding in the striatum as compared to the control group. The authors performed separate experiments to show that the observed decreases in binding were attributable to alterations in the affinity and not the number of binding sites.

Maggos et al. (1998) used PET and SCH 23390 to determine if cocaine-induced D1 receptor changes revert. They used the animal model for the cocaine-binge behaviour described above and determined that D1 receptor binding returned to normal within 10 days. However, when using PET techniques on rats it is important to note that the regions of interest are extremely small such that they may be at the spatial resolution of the camera. This makes exact quantification of tissue radioactivity difficult because it impossible to be sure that neighbouring regions are not being included in the measurement (Hume et al., 1996).

PET studies have not examined D1 receptor changes in humans who abuse cocaine. However, Volkow et al. (1993) used positron emission tomography (PET) and determined that there was a down-regulation of D2 receptor density in the striatum of cocaine abusers. This technology could be used to ascertain if similar neuroadaptions occur with the D1 receptor system.

Rationale for the Present Study

The above review of findings from human and animal research demonstrates that there is justification for research into D1 receptors as a component of reinforcement, relapse and craving in cocaine abuse. These data indicate that D1 receptors may provide a target site for medications aimed at treating cocaine problems.

Repeated cocaine administration is known to lead to chronically high levels of synaptic DA that may result in compensatory changes in DA system. PET has been used to demonstrate a down-regulation of the D2 receptors in cocaine abusers. However, this technology has not been used examine the extent or nature of D1 system changes in these individuals.

In vitro animal studies have shown that there is a decrease in SCH 23390 binding in the limbic regions of rats that have been exposed to cocaine suggesting a down-regulation of D1 receptors (Kleven et al., 1990; Laurier et al., 1994; Moore et al., 1998; DeMontis et al, 1998). Two PET studies conducted on rats using SCH 23390 also suggested a down-regulation of D1 receptors (Tsukada et al., 1996; Maggos et al., 1998).

Based on the above findings, it is hypothesised that PET scans completed with the D1 antagonist SCH 23390 would demonstrate, via a decrease in binding, a down-regulation of D1 receptors in the striatum of human cocaine abusers versus non-users. It was also hypothesised that there would be a positive correlation between the amount, frequency and duration of use of cocaine and the amount of down-regulation evidenced by scans.

Methods

Subjects

Eight cocaine abusers (seven males, one female) were recruited from the Addictions Unit at the Montreal General Hospital (MGH). Subjects were interviewed by a graduate student and a licensed psychiatrist to ascertain DSM IV diagnosis and study suitability. Inclusion criteria were: 1) currently met the DSM-IV diagnostic criteria for cocaine dependence, 2) cocaine use within the seven-day period proceeding the first PET scan, 3) agreement to enter into the clinic's outpatient treatment program, and 4) effective birth control method for women. The exclusion criteria were: 1) current or past psychiatric disorders other than cocaine dependence; 2) current or past use of neuroleptics or other medications that directly act on the dopamine system; 3) current or past neurological disorder, 4) current or past cardiovascular disease; 5) current medical illness; 6) current dependence on a substance other than nicotine or cocaine; 7) pregnancy; 8) under the age of 18 years; 9) previous radiation doses over 5mSv within the past year; 10) the presence of any of the following: a pacemaker, an aneurysm clip, a heart or vascular clip, a prosthetic valve, or a metal prosthesis; and 11) a history of claustrophobia. The Addiction Severity Index was administered to the experimental subjects, urine samples were obtained, and a thorough psychiatric assessment was performed by a psychiatrist. The experimental subjects were matched to eleven control subjects (six male, five female) were nicotine smokers recruited from an advertisement in a local paper for a study that examined the effect of nicotine on D1 receptors using the same radioligand and PET techniques. The control subjects met the exclusion criteria mentioned above. All subjects smoked between half a package and one

package of cigarettes per day. A written consent form was signed by subjects prior to the procedures. The Montreal Neurological Institute (MNI) and the MGH Research Ethics Committees approved the study.

Radiotracer Development

Radiotracers that are labelled with short-lived positron emitters are used to measure the movement of drugs, ascertain metabolic changes, and to map molecular targets such as neurotransmitter receptors. There are positron-emitting isotopes for the natural elements of life: carbon-11 [^{11}C], oxygen-15, nitrogen-13 and fluorine-18; these emitters have a short half-life 21 minutes, 2 minutes, 10 minutes and 110 minutes, respectively. They are produced in a cyclotron and rapidly incorporated into organic compounds in a nearby radiotracer laboratory (Fowler and Volkow, 1998). Carbon-11 [^{11}C] was used to label SCH 23390 for the present study. The radiation dose from a PET study is small because the half-lives of the isotopes are so short that they remain in the body briefly and they are given in very small amounts (Fowler and Volkow, 1998; and Volkow et al., 1991). Radiotracer development is a fundamental aspect of PET methodology because biochemical assessments are based on the utilisation of the appropriate tracer. The data obtained on the distribution of the tracer within tissue are transformed into biochemical information applying a mathematical model (Fowler and Volkow, 1998; and Volkow et al., 1991). Ligands differ with respect to their affinity and specificity for receptors, and in terms of their kinetics.

For the present study, the *Nor*-SCH 23390 free base was first prepared by adding 100 mg of $NaHCO_3$ to 1 mg of *Nor*-SCH-HCl that had been dissolved in 0.5 ml of H_2O. The precipitate was extracted using ether and argon in a warm water bath. The compound obtained was dissolved in 400 µl of dimethyl formamide (DMF) solution. [^{11}C] SCH 23390 was synthesised by N-methylation of the desmethyl precursor SCH 24518 (Schering Plough Pharmaceutical Corporation, Bloomfield, NJ) using [^{11}C] methyliodide (Ravert et al., 1987). The [^{11}C] methyliodide ([^{11}C]-CH_3I was bubbled in the DMF solution at -46^{0}C and then rested for five minutes at room temperature. The solution was heated at 80^{0}C for five minutes. The product was purified on a silica high-performance liquid chromatography (HPLC) (column: Partisil 5 ODS 3, eluant: CH_3CN/0.1 M NH_4COOH 1:1). It was then evaporated and dissolved in saline.

PET and MRI Scans

Positrons travel short distances through tissue before colliding with an electron to generate two annihilation photons (gamma rays) that are simultaneously emitted in opposite directions. The presence of a positron is established by the registration of these two annihilation photons by a series of radiation detectors placed in a circular fashion about the head. The PET camera registers photons that occur in two detectors if they occur within the time-window of the instrument (5-20nsec). The signals are then sent to a computer that stores the data and reconstructs it into an image. The image represents the three-dimensional distribution of the radiocompound (Volkow et al., 1991).

The present study required that each cocaine subject undergo a PET scan within the first week of abstinence from cocaine. A MRI scan was performed on a different day but within the week following the PET scan. All scans were done at the Brain Imaging Centre (PET and MRI Units) of the MNI.

The PET scans were produced with a Siemans ECAT HR+ Scanner that yielded images of 4.2 x 4.2 x 4.0 mm resolution. All subjects (cocaine abusers and controls) were asked to abstain from all psychoactive substances, except nicotine, for at least twelve hours prior to the PET scan. A verbal self-report of substance use during the preceding month was obtained, and it ascertained the date a drug was last used as well as amount, frequency, and route in which it was consumed. Subjects were asked to provide a urine sample to confirm their verbal reports. The urine samples were analysed at the MGH biochemistry laboratory. The laboratory equipment was capable of detecting cocaine within four days after last use.

Prior to the PET scans an intravenous (IV) line was established in the antecubital vein to administer 10 mCi of the radioligand [^{11}C] SCH 23390. The total radiation exposure was less than 5.0 mSv (a dose below Atomic Energy Control Board guidelines). Subjects were positioned in the PET scanner using two orthogonal lasers to correctly align the head. One of the lasers was parallel to the subject's cantomeatal line and the other was parallel to the sagital plane. The head was held in place with a mould (Vac-Lok) that contoured to the shape of the head to restrain movement. Subjects were made as comfortable as possible with pillows for their legs and blankets. The lights were dimmed, noise kept to a minimum and the subjects were asked to relax. A ten-minute transmission scan was performed for the purposes of attenuation correction

using a ^{68}Ge source. The radioligand was then administered in a 120-second bolus and the subjects were scanned for an additional 60 minutes. The scan obtained 63 slices in 26 time frames of increasing duration, such that the initial six frames were taken at 30-second intervals, followed by seven at one-minute intervals, five at two-minute intervals, and eight at five-minute intervals.

A high-resolution (1 x 1 x 1 mm) MRI scan was obtained with a Phillips 1.5T Gyroscan for the purpose of anatomical co-registration. Subjects were given earplugs to reduce the noise from the machine and asked to relax during the 20-minute scan procedure.

Image Analysis

PET and MRI images were transformed into standardised space according to the Talairach-Tournoux Atlas (Talairach, 1988). This atlas provides a series of neuroanatomic sections based on a proportional grid system. The PET frames were summed and co-registered with the MRI image (Evans et al., 1992). The MRI was used as a guide to manually label regions of interest (ROI) on the averaged PET scans. The ROI that were labelled included the NAc, the cerebellum, the right and left CN, and the right and left PU. The cerebellum was labelled as a reference region since it contains a negligible concentration of specific D1 binding (Hall, 1994). The ROI were drawn well within the boundaries of the regions in order to reduce the partial volume effects. The number of consecutive slices labelled for the CN, the PU and NAc was fifteen, ten and three respectively. For the purpose of image analysis, activity in the right and left ROI were averaged.

Time activity curves were extracted and [^{11}C] SCH 23390 binding potential (BP) calculated using the modified method (Gunn et al., 1997) of Lammertsma and Hume (1996). This method of analysis is based on a three-compartmental model that represent the possible environments for the radioligand: 1) blood: unbound or non-specifically bound to plasma proteins; 2) brain tissue: unbound or non-specifically bound to other types of receptors; and 3) brain tissue: specifically bound to D1 receptors. Together these compartments comprise all radiotracer activity captured by the PET. The goal of image analysis is to separate the effects of movement between compartments (i.e., representing the concentration of unbound ligand and non-specific binding) from that of specific binding. BP (B_{max}/Kd), which provides a dimension-less unit, can be expressed as $B_{max} f_2 / K_D^t (1+N_f/K_D^d)$, where is B_{max} the total concentration of D1 binding sites, f_2 is the free fraction of radioligand in the tissue, K_D^t is the equilibrium dissociation constant of the radioligand, N_f is the concentration of free dopamine in the tissue, and K_D^d is the equilibrium dissociation constant of dopamine at the D_1 receptor (Gunn et al., 1997). This analysis method provides a measure of the ratio of blood to brain transfer of tracer in each ROI relative to that in the cerebellum. This ratio is referred to as the ratio index (RI) and it can be taken to be a measure of tracer delivery. The RI is dependent on regional cerebral blood flow (rCBF).

Results

Description of the Sample

The sample for the analyses consisted of eight subjects (seven males and one female) for

the cocaine group. The control group consisted of 11 subjects (six males and five females). As presented in Table 1, 87.5% of the sample was male in the cocaine group versus 54.5% in the control group. All subjects used nicotine. The mean age (±SEM) of the cocaine group was 35 (±2) years and 31 (±3) for the control group. The age range for the cocaine group was 25 - 47 years versus 20 - 52 years for the control group. A t-test indicated that there was no significant difference in the age of the subjects between the two groups ($p>0.05$).

Cocaine Quantity Frequency (CQF) values were calculated for the cocaine group. These values were based upon a detailed drug use inventory and verified by a urine sample as described above. The CQF values (grams/week)were determined by multiplying the number of times that the subject used cocaine in the week prior to the PET scan by the amount in grams of cocaine consumed per session. For the purposes of this study, a session was defined as the time period beginning with the first dose of cocaine and finishing with the final dose of cocaine. Sessions are separated from each other by a period of at least 12 hours. The CQF values ranged from 0.5 g to 10.0 g with a mean value (±SEM) of 4.83 g (±1.18) (see Table 2).

Binding Potentials

Three binding-potential (BP) values were computed for each subject. The values indicated the average binding in the right and left CN and PU, and the NAc. The right and left sides were averaged because it improves the signal to noise and there is no evidence to suggest differences between the two sides. The values represent a relative difference in binding as compared to the cerebellum. Computer-generated time-activity curves and BP values were

obtained for each subject (see Appendix A).

A mean BP was determined for each of the ROI for both the control and cocaine groups. Graphs 1, 2 and 3 display a summary of all data obtained. The means for the three regions were compared between groups using independent t-tests and no significant differences were noted between the groups in any ROI. The mean BP (± SEM) in the CN was 1.55 (± 0.07) and 1.47 (*± 0.09*) for the control and cocaine groups respectively. In the NAc, the BP was 1.14 (± 0.07) for the control group and 1.20 (± 0.08) for the cocaine group. The control group had a BP of 1.66 (± 0.07) in the PU versus 1.60 (± 0.07) for the cocaine group. There was no significant difference in mean BP based on gender (Refer to Table 3).

For the cocaine-abusing subjects, a Pearson correlation determined that there was not a significant relationship between BP in any of the three ROI (i.e. the CN, PU and the NAc) and CQF values or the number of years of problem cocaine use. There was a significant negative correlation noted between age and the BP in the three regions for all subjects as shown in Table 4. Table 5 shows that the mean BP for those that had a problem with cocaine for greater than ten years versus those that had a problem for less than ten years. A t-test showed no significant difference between these means.

Table 1. Age and Gender Stratified by Group

Variable	Cocaine Group (n=8)	Control Group (n = 11)
Mean Age (± SE)	35 years (2.3 years)	31 years (3.2 years)
Gender		
Female (%)	12.5	45.5
Male (%)	87.5	54.5

Table 2. Cocaine Quantity Frequency (QF) Values and Years of Problem Use

Subject Number	CQF (grams/week) (frequency of use) x (quantity used)	Years of Problem Use
1	4.5	0.6
2	2.25	9
3	9	2
4	0.5	1.5
5	6.1	13
6	3.8	10
7	10.00	14
8	2.50	14

Table 3: Mean Binding Potentials in the CN, PU and NAc of Males and Females

Region of Interest	**Mean Binding Potential with SEM (Males)**	**Mean Binding Potential with SEM (Females)**
Nucleus Accumbens	1.16 (± 0.06)	1.16 (± 0.10)
Caudate Nucleus	1.51 (± 0.05)	1.53 (± 0.13)
Putamen	1.62 (± 0.06)	1.67 (± 0.10)

Table 4. **Pearson Correlation for Binding Potentials in CN, PU and NAc and Cocaine Quantity Frequency Values and Years of Use for Cocaine Abusing Subjects, and Age of All Subjects**

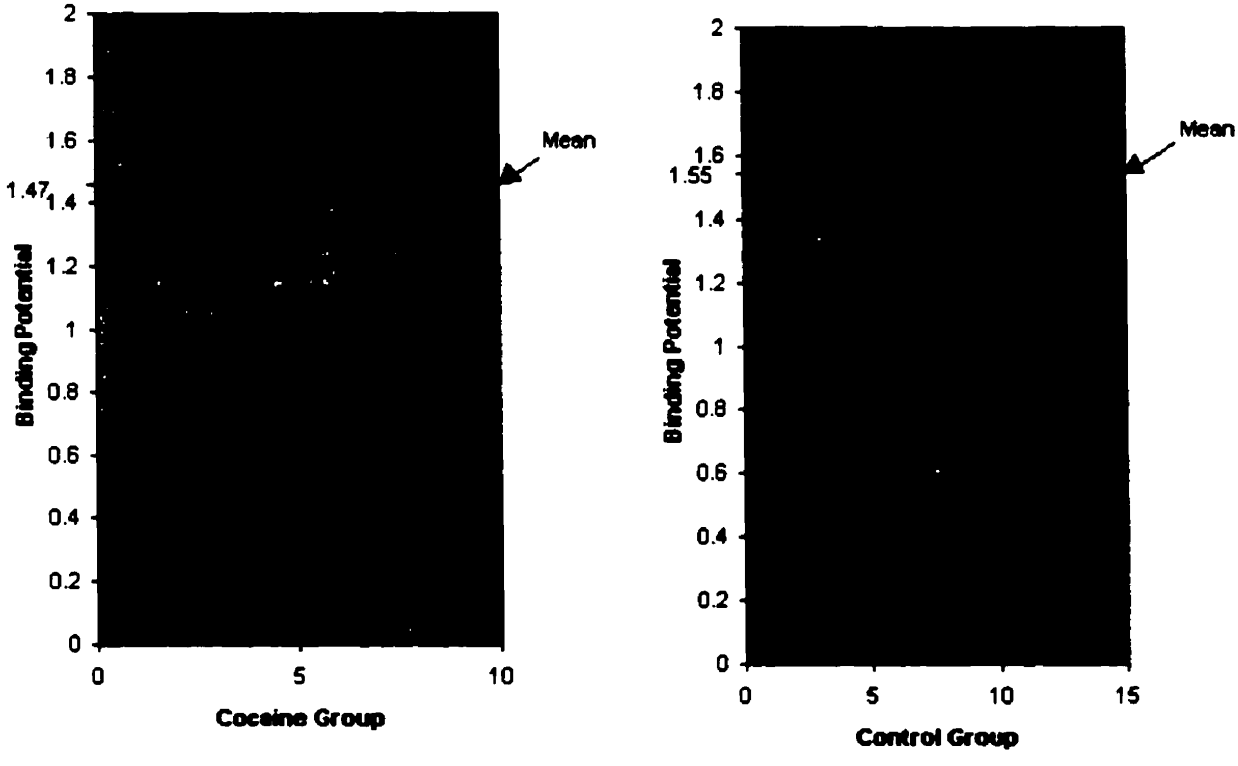
Variable	BP Caudate Nucleus	BP Nucleus Accumbens	BP Putamen
CQF Values (1 week prior to scan)	0.129	0.013	-0.181
Years of Problem Use	0.212	-0.239	-0.076
Age	-0.816*	-0.553*	-0.596*

* $p < 0.05$

Table 5: Mean Binding Potentials in the CN, PU and NAc of Subjects with greater than Ten years versus less than 10 years of Problem Cocaine Use

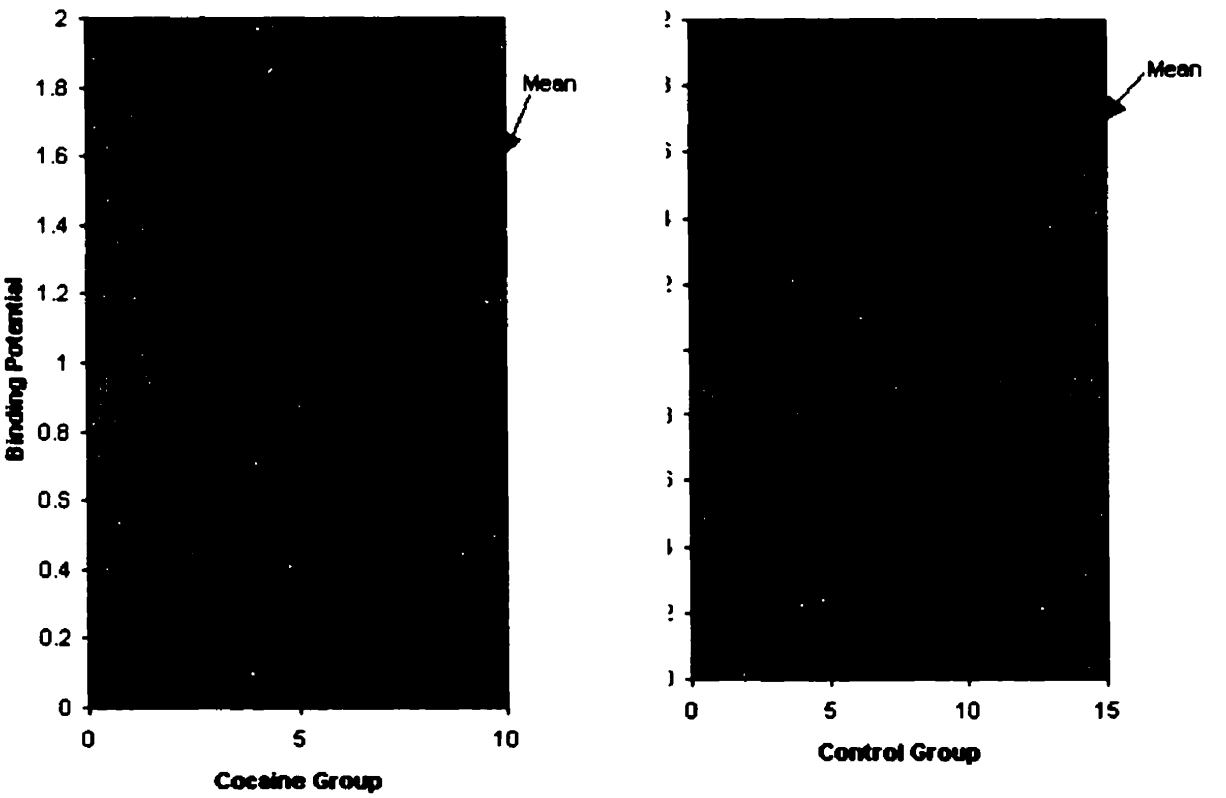
Region of Interest	Mean Binding Potential (Used Cocaine > 10 years)	Mean Binding Potential (Used Cocaine < 10 years)
Nucleus Accumbens	1.21	1.19
Caudate Nucleus	1.53	1.41
Putamen	1.63	1.57

Graph 1. Distribution of Binding Potentials for the Cocaine and the Control Groups in the Caudate Nucleus



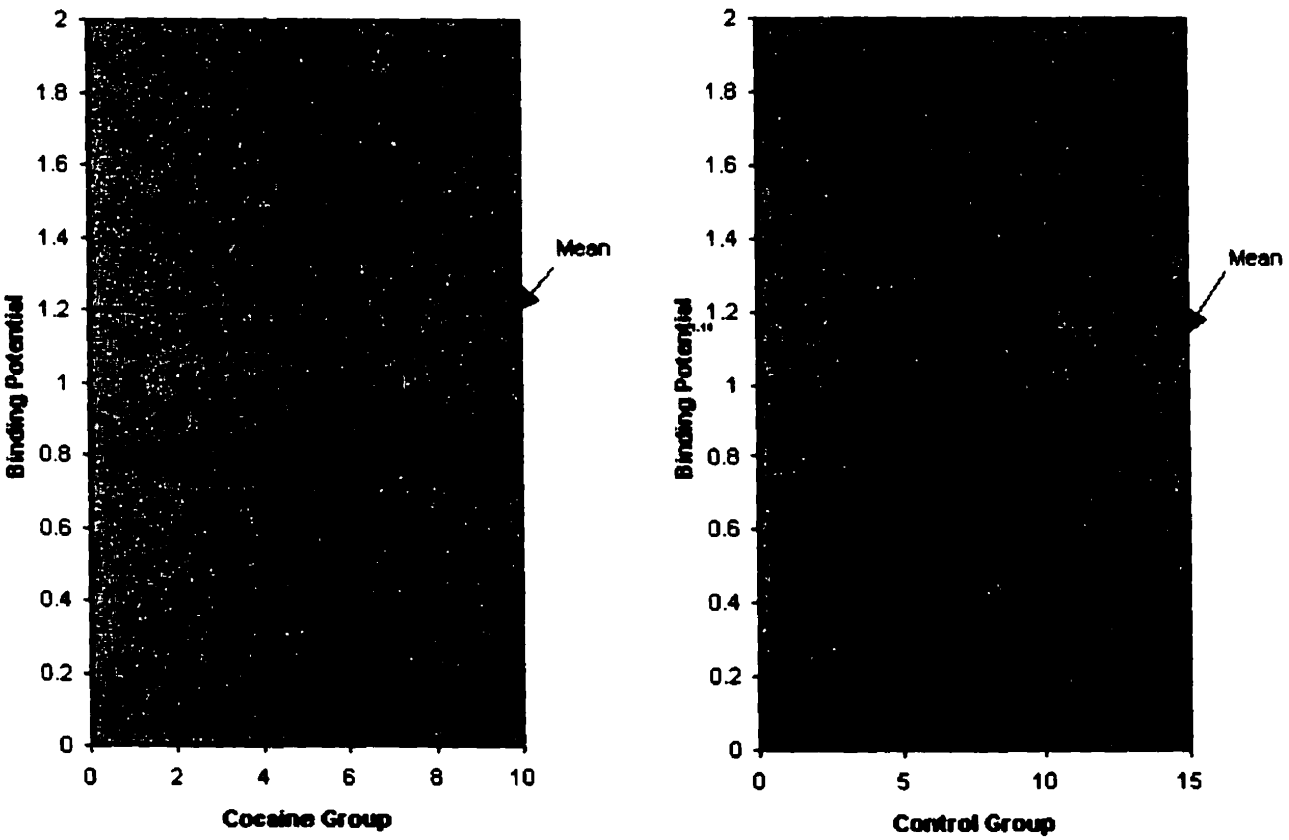
Graph 1 shows the binding potentials (BP) in the caudate nucleus of all subjects. The subjects in the cocaine group had a mean BP of 1.47 versus 1.55 in the control group. The distribution about the mean in the two groups was not significantly different.

Graph 2. Distribution of Binding Potentials for the Cocaine and the Control Groups in the Putamen



Graph 2 shows the binding potentials (BP) in the putamen of all subjects. The subjects in the cocaine group had a mean BP of 1.60 versus 1.66 in the control group. The distribution about the mean in the two groups was not significantly different

Graph 3. Distribution of Binding Potentials for the Cocaine and the Control Groups in the Nucleus Accumbens



Graph 3 illustrates binding potentials (BP) in the nucleus accumbens of all subjects. The subjects in the cocaine group had a mean BP of 1.20 versus 1.14 in the control group. The distribution about the mean in the two groups was not significantly different.

Discussion

The present study attempted to determine if cocaine abuse and dependence leads to neuroadaptions within the dopamine D1 system. The results indicated that D1 receptor binding measured using SCH 23390 was not significantly different between individuals who were using cocaine versus those that had never used cocaine, and that there was no correlation between amount, frequency and duration of cocaine use and binding potential. There was a significant negative correlation with D1 receptor binding and age. This is in agreement with previously reported decreases in D1 receptor density of approximately seven percent per decade in the striatum (Suhara, et al., 1991; Wang, et al., 1998).

Based on a review of the literature, one would expect similar neuroadaptions in D1 and D2 receptors following chronic cocaine dependence; such that if there were a decrease in D2 receptor density there should be a corresponding decrease D1 receptor density. For example, Volkow et al. (1993) used PET technology and found a decrease in D2 receptor numbers in chronic cocaine users (n=7) who were tested within seven days of cocaine administration. The present study found no such decrease in D1 density. However, there are important differences between the studies. Firstly, the Volkow et al. (1993) study did not control for the effects of smoking on DA receptor density. Chronic smoking is thought to alter DA receptor numbers. A PET study using the radioligand SCH 23390 showed decreased binding in the NAc, reflecting a decrease in D1 receptor density (Bleicher et al., 1998). Forty-three percent of the subjects in the Volkow et al. (1993) study were smokers. The number of smokers in their control group was not stated. Therefore, if there were fewer smokers in the control group

versus the cocaine group, smoking could account for the differences in receptor density between groups. The present study included only smokers. Secondly, the average amount of cocaine used per week by the subjects in the Volkow et al. (1993) study was 12.43 grams (g) versus an average amount of 4.83 g in the present study. This difference in average consumption could account for different findings of the two studies. Thirdly, with any comparison of PET findings, one must take into account differing tracer models, the precision and accuracy of the data acquisition, and the assumptions used for image reconstruction and analysis (Volkow et al., 1991). The studies used different methodologies, for example, different radioligands, scanning machines with different resolutions and slice thickness, and they analysed the data with different techniques (i.e. ratio index of two images obtained at 11 and 114 minutes post-injection versus three-compartmental model using 26 averaged PET images). The studies also differed in the size and shape of their ROI. Precise location of an anatomical area on a PET image is extremely difficult not only because of the lack of precise boundaries among brain areas but also because of the normal anatomical variability among individuals. In general, the smaller the ROI the more imprecise its location, particularly as it approaches the resolution of the instrument (Volkow et al., 1991). The present study used co-registered MRI images to verify location on the PET image and the Volkow et al. (1993) study did not employ this technique. The numerous technical variations between the studies make it impossible to compare and comment on the different findings.

Maggos et al. (1998) used PET technology to examine rat brains. They reported that D1 receptor binding was decreased following chronic cocaine use but that it had returned to

normal levels within ten days of withdrawal. An additional finding of this study was decreased D2 receptor binding that persisted at ten days. Thus, it appears that normalization of D1 receptors occurs more quickly than it does for D2 receptors. If changes in D1 receptor density do occur in humans, the time frame for normalization is not known. The subjects in the present study may have been examined after their D1 receptors had returned to normal levels.

In vitro animal studies have found that chronic cocaine administration can induce a decrease in the number of D1 receptors (Kleven et al., 1990; Laurier et al., 1994; Moore et al., 1998; De Montis et al., 1998). There are several key points to consider when comparing the effects of cocaine on animals and humans. Humans and animals are studied under markedly different conditions, for example, rigid laboratory environment versus naturalistic techniques. The *in vitro* studies mentioned above utilized stringent methodologies such that the investigators knew exactly how much cocaine was administered over a specific duration and pattern. The present study did not control cocaine administration and there was a great deal of variability within the cocaine group in terms of CQF values (i.e. 0.50 – 10.0), number of years of problem use (i.e. 6 months – 14 years), and patterns of use (i.e. daily versus binging). Although animal investigators attempt to mimic human drug use patterns, there is no gold standard for their replication. It is likely that amount, duration, and patterns of cocaine use significantly effect how neurotransmitter systems adapt. The route of administration for the animal studies mentioned above included intraperitoneal and intravenous injection whereas the subjects in the present study either inhaled or smoked cocaine. The route of administration

alters the pharmacokinetics of a drug and this again may alter the brain's physiological response (Sellers et al., 1991).

In addition, there are important differences between species in terms of physiological responses to drugs and brain anatomy. For instance, intravenous cocaine administration in primates results in decreased brain metabolism (Lyons et al., 1996), whereas it increases metabolism in rats (Porrino et al., 1988). Therefore, neurochemical responses in animals may not reflect neurochemical responses in humans. It is likely that *in vitro* and *in vivo* techniques could come to different conclusions.

A number of factors are known to effect the outcome of PET studies and include such things as age, sex, handedness, IQ, body weight, time from last drug exposure, dietary and lifestyle choices (Gatley & Volkow, 1998). Although the present study assessed age, sex and the time since last use, the other factors were not assessed. To improve study design subjects should be matched on characteristics that are known to effect outcome.

A more general explanation for the lack of differences between the experimental and control groups in the current study rests on the validity of the DA hypotheses of cocaine addiction. This hypothesis is well supported by animal studies but it has not been confirmed by human studies. Because DAergic agents are readily self-administered in animals, the DA hypotheses predicts that they will produce euphoria and have abuse potential in humans. However, clinical observation has revealed inconsistencies between humans and animals.

Andersen (1987) found that four widely prescribed DA re-uptake inhibitors bupropion (Wellbutrin™), nomifensine (Merital™), mazindol (Sanorex™), and benztropine (Cogentin™)

were more potent than cocaine in inhibiting [^{3}H] DA uptake into striatal synaptosomes yet they were not abused by humans. Wong et al. (1993) demonstrated that an oral clinical dose of one of these agents, mazindol, produced significant occupation of the DAT in the mesolimbic region. This helped to eliminate the explanation that this group of drugs was not occupying DAT in the appropriate brain regions. GBR 12909, an antidepressant used in Europe, is several hundred times more potent that cocaine in inhibiting [^{3}H] DA uptake and PET studies found that it occupied DAT at low doses (Kilbourn et al., 1989). It is clear from the literature that euphoria is not a primary effect or a side effect of DA re-uptake inhibitors and they are not abused (Rothman and Glowa, 1995). The situation is similar with another group of DAergic agents, the DA agonists. Pergolide and bromocriptine are DA agonists used to treat Parkinson's disease, acromegaly, and hyperprolactinemia. Woolverton et al. (1984) found that these agents are self-administered in animals but clinical observation demonstrates that they do not produce euphoria in humans (Rothman and Glowa, 1995). Drugs that are known to increase synaptic concentrations of DA, such as monoamine oxidase (MAO) inhibitors and L-DOPA, should have a cocaine-like effect. Again, animals have been trained to self-administer DA enhancers (Colpaert et al., 1980), but they have not been abused in humans (Rothman and Glowa, 1995). DA antagonists that are prescribed as antipsychotics in humans have been found to attenuate cocaine self-administration in animals. Clinical practice demonstrates that antipsychotic medications do not necessarily attenuate the euphoric effects of cocaine in schizophrenic patients (Dixon et al., 1991).

There have been explanations for the above clinical observations. For example, Rothman and Glowa (1995) argue that there is no precise way of determining a drug's reinforcing capability through animal paradigms. For instance, if an animal increases its rate of lever-pressing following administration of a pharmacological agent, does that represent an increase or a decrease (potentially due to a partial blockade of receptors) in the reinforcing effects of the drug. Second, numerous data suggest that the mesolimbic DA has general, non-specific functions with respect to different goal-directed behaviours that are established by natural and drug reinforcers (Ljunberg et al., 1992; Koepp et al., 1998). It is likely that the pattern of mesolimbic DA activity seen to accompany cocaine self-administration in animals is similar to other goal-directed behaviours. These observations point to the need for human studies and suggest that the DA hypothesis may be too simple to completely explain the effects of cocaine in humans.

Neurochemical systems that have a close functional relationship with DA are affected by cocaine. For instance, the order of cocaine's potency in the inhibition of monoamine re-uptake is highest for 5-HT followed by DA and NA. It has been suggested that these three systems are involved in the mediation of pyschogenic effects of cocaine, as well as in the development and regulation of drug taking. Thus, mesolimbic DA activity may be one component of a closely interrelated and interdependent system affected by cocaine (Kiyatkin, 1994).

PET studies have been used to examine neurochemical adaptations in other neurotransmitter systems. Wang et al. (1995) determined that there was no change in 5-HT2

receptor numbers in chronic cocaine abusers. However, Zubieta et al. (1996) examined μ-opioid binding in cocaine-dependent men using PET and [^{11}C]carfentanil. They found that specific binding was increased in several brain regions of the addicts studied one to four days after their last use of cocaine. Binding was positively correlated with the severity of cocaine craving. Up-regulation of μ-opioid-receptor binding persisted after four weeks of monitored cocaine abstinence. These finding suggest involvement of the endogenous opioid system in cocaine dependence and cocaine craving. More PET studies are required to examine the interactions between systems that are influenced by cocaine.

Although animal research has supplied crucial data about the cellular and molecular mechanisms underlying cocaine actions, it is important to conduct human studies. PET technology permits the visualisation of the neurochemical consequences of chronic cocaine use, something that is not possible to ascertain from animal research.

To conclude, based on the present study it is not possible to say conclusively whether changes in D1 receptor density occur with chronic cocaine use. More subjects need to be examined with greater control over factors that may affect outcome (such as time since last cocaine dose). It would be of interest to ascertain if higher CQF values and more years of problem use would alter the results. In addition, a longitudinal study that documented changes from abnormal towards normal during recovery from dependence would provide valuable information that would prove useful in the development of appropriate pharmacotherapy.

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Appendix A

Time-activity curve versus binding potential
for one subject