Cocaine Cue-Induced Dopamine Release in Amygdala and Hippocampus: A High-Resolution PET [^{18}F]Fallypride Study in Cocaine Dependent Participants

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ترکیب طبایع چو به کام تو دمی است روشاد بزی اگرچه برتو ستمی است

با اهل خرد باش که اصل تن تو گردی و نسیمی و غباری و دمی است

"خیام"

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ABSTRACT

Background: Drug related cues are potent triggers for relapse in people with cocaine dependence. Dopamine release within a limbic network of striatum, amygdala and hippocampus has been implicated in animal studies, but in humans it has been possible to measure effects in the striatum only. The objective here was to measure dopamine release in the amygdala and hippocampus using high-resolution PET with [^{18}F]fallypride.

Methods: Twelve cocaine dependent volunteers (mean age: 39.6±8.0; years of cocaine use: 15.9±7.4) underwent two [^{18}F]fallypride HRRT PET scans, one with exposure to neutral cues and one with cocaine cues. [^{18}F]Fallypride non-displaceable binding potential (BP_{ND}) values were derived for five regions of interest (ROI) (ventral limbic striatum, associative striatum, sensorimotor striatum, amygdala, and hippocampus). Subjective responses to the cues were measured with visual analog scales and grouped using principal component analysis.

Results: Drug cue exposure significantly decreased BP_{ND} values in all five ROI in subjects who had a high but not low craving response (limbic striatum: p=0.019, associative striatum: p=0.008, sensorimotor striatum: p=0.004, amygdala: p=0.040, and right hippocampus: p=0.025). Within the striatum, individual differences in the cue-induced craving response predicted the magnitude of [^{18}F]fallypride responses (ventral limbic: r=0.581, p=0.048; associative: r=0.589, p=0.044; sensorimotor: r=0.675, p=0.016).

Conclusions: To our knowledge this study provides the first evidence of drug cue-induced dopamine release in the amygdala and hippocampus in humans. The preferential induction of dopamine release among cue-responders suggests that these aspects of the limbic reward network might contribute to drug seeking behavior.

ABRÉGÉ

Historique: L'indice lié à la drogue est un puissant élément déclencheur pour une rechute chez les gens avec une dépendance à la cocaïne. La libération de la Dopamine dans le réseau limbique du striatum, les amygdales et les hippocampes a été démontrée dans les études animales, mais avec l'humain, il a été possible de mesurer les effets spécifiques au striatum. Notre objectif est de quantifier le relâchement de dopamine dans les amygdales et hippocampes en utilisant la TEP à haute résolution avec injection de [^{18}F]fallypride.

Méthode : Douze bénévoles dépendants de la cocaïne (moyenne d'âge : 39.6±8.0; nombre d'années d'utilisation de la cocaïne : 15.9±7.4) ont subi deux scans HRRT TEP avec [^{18}F]fallypride: Une avec exposition de l'indice neutre et l'autre avec l'indice de la cocaïne. Les valeurs du potentiel de fixation (BP_{ND}) du [^{18}F]fallypride sont tirées de cinq régions d'intérêt (RDI) (striatum limbique ventral, cortex associatif, striatum sensorimoteur, amygdales et hippocampes). Les réponses subjectives de l'indice déclencheur sont mesurées avec des échelles analogues visuelles et groupées à l'aide d'analyses en composantes principales.

Résultats : L'exposition de l'élément déclencheur de la drogue diminue significativement les valeurs $BP_{ND\,d}$ aux cinq RDI des sujets qui ont eu un désir élevé de consommer (striatum limbique p=0.019, cortex associatif : p=0.008, striatum sensorimoteur : p=0.004, amygdales : p=0.040 et l'hippocampe droit : p=0.025). A l'intérieur du striatum, les différences individuelles dans la réponse à la provocation de l'élément déclencheur prédisait l'ampleur des réponses [^{18}F]fallypride (limbique ventral: r=0.581, p=0.048; cortex associatif: r=0.589, p=0.044; sensorimoteur: r=0.675, p=0.016).

Conclusion: À notre connaissance, cette étude apporte la première preuve que l'élément déclencheur provoque le relâchement de la dopamine dans les amygdales et hippocampes chez les humains. L'induction préférentielle du relâchement de la dopamine parmi les répondants aux indices de la drogue suggère que ces aspects du réseau limbique pourraient contribuer aux comportements de recherche de la drogue.

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Contribution of Authors

McGill University requires that in the case where a multi-author manuscript is included in a thesis an explicit statement of the contribution of the authors is included.

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AF coordinated the study and was responsible for data collection, analysis and interpretation, as well as the initial draft of the manuscript. ML conceived of and designed the study, with input from AF, CB, KFC and SMLC. AR, KL, JV and PG contributed to collection and analysis of imaging data. All authors contributed to critical edits of the manuscript.

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General Introduction

Problem of Addiction

Socioeconomic Impact

Substance use and abuse is a growing threat to public health that is associated with severe social, familial, medical and economic problems. It imposes large costs on the health care system and has been one of the major public health concerns in many countries for decades.

The prevalence of drug abuse is very high, as is the associated mortality and morbidity. According to the 2011 World Drug Report (WDR), the past decade has witnessed an increase in the number of people[1] who use illicit drugs, from 180 million to 210 million worldwide. This statistic represents approximately 5% of the world population and reflects a population with higher mortality and heightened exposure to various health risks. In 2011, the United Nations Office on Drugs and Crime estimated that the mortality rate due to illicit drug use was between 23.1 and 58.7 deaths per million people, increasing up to more than 3 fold (148 deaths per million) in North America. This high mortality rate reflects the higher susceptibility to a large number of health conditions and risky behaviors, including fatal drug overdoses, suicides, accidents, contraction of HIV/ AIDS and hepatitis C, and multiple other medical conditions (e.g. organ failure) (UN Office on Drugs and Crime, 2011).

The high prevalence of illicit drug use is associated with increased social and economic burden. According to the National Institute of Drug Addiction (NIDA), a large portion of social problems including violence, child abuse and accidents are precipitated and aggravated by substance abuse. As a result, huge financial burdens are placed on the government as a result of the necessary social services, relevant organizations, specialized care and appropriate treatments. In the United States, the costs associated with substance use add up to more than $484 billion per year (National institute on Drug abuse, 2012). In Canada alone, it is associated with an estimated $40 billion in economic

[1] 15-64 years old

costs per annum (Rehm et al., 2006). These concerns are heightened given that the affected subpopulation includes young adults, students, pregnant women and fulltime employees; all prolific populations in most societies (Sinha, 2011).

Cocaine is among the most widely used illicit drugs and ranks fourth in terms of global prevalence (UN Office on Drugs and Crime, 2011). According to WDR, 0.3-0.5% of the world's adult population[2] use cocaine. This percentage is equivalent to 14 to 21 million users. The highest concentration of cocaine users is found in North America, comprising 37% of all cocaine users worldwide and about 2% of North America's population. According to the Canadian Addiction Survey (Adlaf, 2005), 10.6% of respondents aged 15 and up reported having used cocaine in their lifetime, a significant increase from 3.8% in 1994 (Rehm et al., 2006). Treatment success for cocaine dependence is low, and relapse rates are high. In North America, cocaine is one of the three most harmful drugs as reflected in treatment demands; in South America, it is the primary drug problem for which people seek treatment (UN Office on Drugs and Crime, 2011). These statistics highlight cocaine, among other drugs of abuse, as a drug that requires special attention from health policy makers and planners.

Despite the aforementioned problems, available treatments for substance use disorders yield only modest success and treatment failure rates continue to be very high. On average, 85% of drug dependent users relapse in the first year after treatment (Sinha, 2011). The proposed treatments encompass a variety of pharmacological and behavioral interventions, each based on a distinct hypothesis to explain addiction. These hypotheses include biological and psychological perspectives. Proposed biological hypotheses encompass those that suggest immunologic mechanisms leading to the development of a cocaine vaccine (Orson et al., 2009, Shorter and Kosten, 2011), impairment of the electrical activation of the brain justifying the use of anticonvulsants (Alvarez et al., 2010) and alterations in brain neurochemical circuits supporting the use of anti-dopaminergic medications (Carroll et al., 1999). In comparison, cognitive behavioral treatment approaches include contingency management therapies to redirect goal seeking (Higgins and Petry, 1999), as well as cognitive therapies to improve insight and motivation (Carroll et al., 1991).

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[2] 15-64 years old

To date, no single treatment has been able to conquer the high rates of relapse. This reflects, in part, the poor understanding of the underlying mechanisms through which addiction forms and sustains. A better understanding of addiction as a disease is likely an important step towards finding promising and cost effective treatments.

In summary, the high and increasing rates of drug use, along with the associated social and economic problems and lack of effective treatments, underscore why substance abuse has become such a prominent issue in society. Additionally, it highlights the need to unravel the underlying mechanisms of substance dependence, particularly for illicit drugs with high prevalence rates such as cocaine.

Symptom Profile

Drug addiction is a compulsive engagement in drug use that affects all aspects of an individual's life. The individual spends large amounts of money and a great portion of time finding and consuming drugs. These practices lead to reduced time spent on other activities, including social, personal and professional responsibilities. Attempts to quit, along with a persistent desire to cut down or quit, are common but frequently unsuccessful. A vicious cycle forms consisting of repeated attempts to quit and failing to maintain abstinence, a situation that defines relapse (American Psychiatric Association, 2000). This persisting susceptibility to discrete bouts of drug use, alongside the progressively narrowing focus on drug use, constitutes the core features of addiction (Brownell et al., 1986).

Individual episodes of drug use are inevitably preceded by exposure to drug related cues – people, places, paraphernalia (Self, 1998). These cues can elicit intense motivational states and narrowed attentional focus which can be accompanied by alterations in subjective, behavioural and physiological states, e.g. intense craving and autonomic arousal (Ehrman et al., 1992, Childress et al., 1993). These elicited states can lead to drug seeking behavior and subsequent drug relapse (Carroll et al., 1991, Dackis and O'Brien, 2001). Understanding the mechanisms and triggers of relapse might be a promising approach towards treatment or even prevention of severe addictions.

Theories of drug addiction and relapse

The mechanisms of addiction have been debated for decades and more. The two most influential hypotheses propose that drug-seeking behavior is a manifestation of avoiding aversive effects of withdrawal (*e.g.,* (Wikler, 1948, 1973)) or a drive to seek out reward (*e.g.,* (Vogel et al., 1948, Robinson and Berridge, 1993)). Although there is a general consensus that the negative states of withdrawal contribute to vulnerability to relapse (Koob and Moal, 1997, Hutcheson et al., 2001), this is unlikely to be the primary mechanism underlying drug seeking behavior (Vogel et al., 1948). For example, drug withdrawal *per se* does not elicit drug-seeking behavior (Stewart, 2008), nor is it necessary for relapse; indeed, a common cause of drug-related mortality is the re-initiation of drug use following an extended period of abstinence, long after withdrawal symptoms – and tolerance – have dissipated (Merrall et al., 2010). In both laboratory animals and humans, high levels of drug self-administration can develop in the absence of ever having experienced withdrawal symptoms (Vogel et al., 1948, Stewart et al., 1984).

A substantial body of research indicates that individual bouts of drug relapse are mainly precipitated by reward seeking processes. For example, in laboratory animals drug seeking behaviors can be potently induced by exposure to small amounts of the drug and drug related cues (de Wit and Stewart, 1981, Stewart et al., 1984); see also (Pickens and Harris, 1968, Gerber and Stretch, 1975, Davis and Smith, 1976), but not by the induction of withdrawal symptoms (Stewart, 1983). These effects are the opposite of what would be predicted if drug seeking was primarily due to withdrawal or other physiological deficits. Secondly, volitional electrical brain stimulation of these regions simulates aspects of addiction. In these studies, animals developed compulsive self-stimulation behaviors such that they ignored natural rewards and continued to engage in self-stimulation behaviors even if they led to punishment (Olds and Milner, 1954, Olds, 1958, Routtenberg and Lindy, 1965). Third, in laboratory animals lesions to brain regions associated with reward (e.g. ventral tegmental area, VTA) disrupt appetitive responses for cocaine (Twining et al., 2005). Together, these observations support the proposition that core addiction like behaviors could reflect disturbances of reward processes (de Wit and Stewart, 1981, Stewart et al., 1984).

Reward Neuroanatomy

The transmitter system most clearly implicated in responses to rewards and reward related cues is the ascending midbrain DA system. DA cell bodies in the substantia nigra (SN) and VTA project to cortical and limbic regions forming the mesocortical and mesolimbic pathways. The mesolimbic DA system mainly includes projections from the VTA to the NAcc, olfactory tubercle and the most ventral parts of caudate and putamen, as well as the septum, amygdala and hippocampus. The mesocortical DA system consists of dopaminergic neuronal extensions from the VTA to cortical regions such as orbitofrontal cortex (OFC), prefrontal cortex (PFC) and cingulate gyrus (Haber and Knutson, 2010). Nigro-striatal DA cells project from the substantia nigra, pars compacta (SNc) to the dorsal striatum.

The ventral striatum (VS) receives inputs from limbic cortex (e.g. ventromedial PFC, OFC, cingulate) as well as subcortical regions such as the amygdala and hippocampus. The central striatum, in comparison, receives negligible input from the hippocampus and amygdala, and progressively less input from limbic cortex, but more input from associative cortex. This gradient of changes continues to the most dorsolateral aspects of striatum which receives input from sensorimotor cortex. Efferent projections from the striatum descend back to the midbrain DA cell bodies in a series of spiralling midbrain-striatal-midbrain loops, allowing for the transfer of information across the striatal gradient which may represent a mechanism by which motivation is translated into action (Mogenson et al., 1980, Haber et al., 2000, Martinez et al., 2003, Haber and Knutson, 2010, Ikemoto, 2010).

Though these regions typically function as an integrated network, each area is preferentially involved in different processes. The VS is particularly implicated in the initial acquisition of a response to novel rewarding stimuli, whereas the dorsal striatal regions, including (posterior) caudate nucleus and putamen, are thought to contribute to habit formation (Yin and Knowlton, 2006). Striatal regions can also be functionally classified as limbic, associative and sensorimotor striatum. The limbic striatum consists mainly of the ventral striatum and is involved in motivation; the associative striatum

includes the pre-commissural and both pre and post commissural[3] caudate and is involved in cognition; and the sensorimotor striatum is centered in the posterior putamen and is involved in locomotion (Martinez et al., 2003).

As noted, the hippocampus and amygdala also receive DA input from the VTA and are thought to influence aspects of reward processing including the responses to motivationally important cues (Tracy et al., 2001, Tye and Janak, 2007), formation of memory and associated learning of reward and reward predictors (Robbins et al., 2008), and the development and expression of habit-like behaviors (Lingawi and Balleine, 2012). Extensive network connectivity of the amygdala and hippocampus with other reward regions (e.g., NAcc, Ventral Subiculum (VSub) and medial PFC) is suggestive of this modulating role. For example, a substantial portion of NAcc inputs come from the amygdala (Heimer et al., 1997) and efferent projections of central nucleus of the amygdala are known to be major modulators of DA transmission in the SNc and VTA (Fudge and Emiliano, 2003). The hippocampus is also innervated by dopaminergic afferent fibers from the VTA and amygdala, and its efferent fibers project to striatal regions such as the NAcc. More specifically, the ventral CA1 and subiculum project to the NAcc shell whereas the dorsal CA1, subiculum and parahippocampal regions innervate the NAcc core (Fanselow and Dong, 2010). These neuronal projections (from the hippocampus to the NAcc) are reported to elicit a DAergic response in the NAcc (Floresco et al., 2001). Interestingly, hippocampus and amygdala have been reported to be highly interconnected, allowing a mutual modulation of synaptic plasticity (Maren and Fanselow, 1995, Akirav and Richter-Levin, 2002). Szalay et al. suggest that the dorsal SUB and basolateral amygdala (BLA) interact serially and convey contextual and emotional information to the NAcc, where they are integrated and assign salience to the reward predicting cues (Szalay et al., 2011). Moreover, Burns et al. argue that the behavioral response to drug cues involves ventral subiculum - ventral striatum - BLA-ventral striatum pathways. In their study approach to the stimuli predictive of sucrose reward as well as the ability to acquire a new response with conditioned reinforcement were disrupted by lesioning either of these pathways (Burns et al., 1993).

[3] Precommissural and postcommissural refer to rostral and caudal to the anterior commissure

Together the above findings suggest that the amygdala, hippocampus and striatum form a neural network that regulates multiple aspects of reward processing (Haber and Knutson, 2010, Sesack and Grace, 2010).

Neurobiology of Reward and addiction

Dopamine, Natural and Drug rewards

Reward is a multi-faceted concept (attention, learning and approach) likely involving the contribution of various neurotransmitters, including glutamate, norepinephrine, serotonin, endogenous opioids and GABA (Koob and Le Moal, 2001, Berridge and Robinson, 2003, Ross and Peselow, 2009). However multiple lines of evidence suggest that DA plays a particularly important role for at least some aspects of reward (Wise and Rompre, 1989, Schultz, 1997, Berridge and Robinson, 2003, Berridge, 2007).

Among the first observations that supported this proposition were that, in laboratory animals, lowered DA neurotransmission disrupted behavioural responding for electrical stimulation of the brain (Franklin and McCoy, 1979), stimulant drugs (Yokel and Wise, 1975) and food (Wise et al., 1978). Subsequent microdialysis studies demonstrated that providing natural rewards such as food (Hernandez and Hoebel, 1988, Martel and Fantino, 1996) and sex (Meisel et al., 1993) can lead to a marked increase in DA level in the VTA and NAcc. In humans, consumption of food (Small et al., 2003) or engaging in rewarding tasks such as playing video games with monetary reward (Zald et al., 2004) are reported to elicit a heightened DA response in the striatum. Furthermore, findings from both animal and human studies support the association between behavioral responses to rewarding cues and a heightened striatal DA signal. For example, exposure to reward paired cues (such as food cues) has been shown to induce a DA response in brain reward regions (e.g. NAcc) in rats (Phillips et al., 1993, Schultz, 1998, Nakazato, 2005, Cacciapaglia et al., 2012). In humans, exposure to food stimuli, e.g. watching pictures of food or smelling odors related to food, induced a striatal DA response (Volkow et al., 2002, Wang et al., 2011). These observations indicate that the response to reward and reward associated cues similarly involves DA transmission, and the behavioural response could also share similar features.

In line with the body of literature that reports DA signalling is involved in response to natural reward, several lines of evidence suggest that drugs of abuse also owe their rewarding properties to alterations in transmission of DA in the reward pathway (Wise, 1996). First, the best established pharmacological property that nearly all addictive drugs share is functional DA agonism (Di Chiara and Imperato, 1988, Lowinson et al., 2005). Microdialysis methods reliably demonstrate increased DA release in brain reward regions such as the NAcc when drugs are self-administered in animals (Wise, 1993, Wise et al., 1995). Second, the administration of DA antagonists in animals with established self-administration behavior disrupts the self-administration of stimulant drugs (Yokel and Wise, 1975). In humans, explicit investigation of pathways and neurotransmitters associated with drug use has been carried out by imaging studies (e.g., PET) that target DA directly. These studies showed that the administration of stimulant drugs such as cocaine, amphetamine and methylphenidate increase DA transmission in the striatal regions (Volkow et al., 1999, Leyton et al., 2002, Cox et al., 2009). Collectively, these observations suggest that the role of DA signal in influencing rewarding aspects of drug of abuse is significant and needs to be further elaborated.

In accordance with the findings that highlight DA neurotransmission in response to natural reward predicting stimuli, recent studies indicate that exposure to drug cues also increases DA signalling in mesocorticolimbic pathways. Several lines of evidence are in favor of this proposition. First, animal studies have demonstrated that drug cues are able to elicit and augment DA responses in the striatum and amygdala (Weiss et al., 2000). Second, alterations in DA transmission by DA agonists or antagonists affect cue induced drug seeking behavior (Vorel et al., 2002). Third, recent neuroimaging studies provide evidence of drug cue-induced DA neurotransmission in the in human striatum. In healthy subjects, exposure to a regimen of repeated amphetamine administration can lead to the ability of the drug paired cues to induce DA release in the ventral striatum (Boileau et al., 2007). In cocaine dependent users, exposure to drug paraphernalia and images induces a DA response in dorsal striatum (Volkow et al., 2006, Wong et al., 2006). This cue-induced DA release may have functional significance since decreasing DA transmission diminishes cocaine cue-induced craving (Berger et al., 1996, Leyton et al., 2005).

Taken together, these observations suggest that rewarding aspects of drug use and responses to drug cues might be regulated through neurotransmission of DA and highlight the importance of further investigation of DA's role in these processes.

Roles of dopamine in reward learning, prediction, and motivation

Although there is consistent evidence that addictive drugs and drug related cues can activate at least some aspects of the mesocorticolimbic DA pathways, the behavioral significance of these activations remains a subject of debate. Two theories of DA's involvement in reward that have particular relevance for the current thesis and the "incentive salience" and "learning and memory" hypotheses (Di Chiara, 2002, Berridge, 2007).

The "DA learning hypothesis" of reward suggests that DA acts as a communication signal that connects neuroanatomical memory circuits (such as the striatum, frontal regions, amygdala and hippocampus) and contributes to learning processes associated with the acquisition, consolidation and retrieval of reward experience and its predictors (Di Chiara, 2002). This hypothesis in fact consists of several different views on how DA modulates reward learning that are closely related. First, DA has been proposed as a learning signal that contributes to the strengthening of associations between S–R (stimulus–response) or S–S (stimulus–stimulus) and regulates the "stamping-in" of these memory links. Support for this proposition is mainly provided by the experiments that showed pharmacological manipulation of DA levels affects the learning associated with reward and their stimuli. For example, administration of DA antagonists before and after a reward learning task can disrupt acquisition and consolidation of the associations respectively (Wolterink et al., 1993, Kelley, 2004). Furthermore, DA agonists administered at the time of training enhanced the learning of the association between reward and cue (that is, rats learned to work more for the cues) (Robbins and Everitt, 1996). These findings are congruent with the molecular biology data that propose a critical role for DA in modulation of neuronal synaptic plasticity. These studies suggest that DA can affect long-term potentiation (LTP) and depression (LTD) of neuronal synapses in the memory circuits. Although other neurotransmitters (specifically glutamate) are also implicated in regulation of synaptic plasticity, pharmacological studies using DA agonists or antagonists have provided evidence that

LTP and LTD are highly associated with DA signalling in brain regions that are mostly implicated in learning and memory processes such as the hippocampus (Frey et al., 1990, Otmakhova and Lisman, 1998), amygdala (Bissiere et al., 2003), frontal cortex (Otani et al., 2003) and VTA (Bonci and Malenka, 1999). Taken together, this view suggests DA as a signal that mediates reward's associative "stamping in" presumably by influencing cellular and molecular plasticity.

Secondly, an extended version of the first viewpoint highlights DA's role in "habit learning" and "habit performance" aspects of reward learning. This view point suggests that DA serves a strengthening function that can mediate (i) establishment of a stronger than normal learned S-R that leads to habit performance, that is, persistence of behavior even when reward is devalued (e.g. by aversive conditioning) and (ii) habituation of action patterns that are never learned as an S-R. For example, some action patterns (such as novel combination of motor stereotypy) can become 'habitual' the first time that high doses of amphetamine are administered (Sahakian et al., 1975). These observations suggest that DA might have a distinct role in habit learning.

Another learning theory of DA proposes DA as a "prediction error signal of reward". Schultz et al. conducted a series of pioneering experiments in which neuronal DA bursting was recorded when the monkey was presented with reward signalling cues (Schultz et al., 1997). They report that DA neuronal firing was correlated with the accuracy that cue could predict actual reward. These findings are only correlations, however, they suggest that DA, as a learning signal, might be involved in modulating the associative connections between reward and its predictor signal. Collectively, the learning theory of DA in reward is supported by behavioral studies and cellular and molecular experiments indicating that DA plays an important role in the formation and retrieval of the memory of a rewarding event and its associated stimuli.

Despite the compelling evidence that DA is involved in numerous reward related memory processes, some of these processes appear to be independent of DA transmission. For example, DA deficient mice are still able to learn the location of rewards (Cannon and Palmiter, 2003, Hnasko et al., 2005, Robinson et al., 2005). Moreover, very recent work suggests that DA's contribution to reward learning might occur specifically when incentive salience is attached to the reward paired cue: rats that

fail to imbue the cue with incentive salience can still learn the association but do not exhibit accumbens DA release in response to the cue (Flagel et al., 2011). Taken together, these data suggest that although DA transmission might influence some aspects of learning, this does not provide sufficient explanation of all the mechanisms associated with behavioural response to rewards and reward related stimuli. Hence, our interpretation of DA's contribution to reward learning should be addressed considering other aspects of DA's function in regulating these processes.

A second influential hypothesis proposes that limbic DA influences reward seeking behaviors primarily by changing the ability of reward related cues to grab and hold attention (Berridge, 2007). In support of this view, pharmacological alterations of DA transmission alter various reward related behavioral responses. For example, in laboratory rats, pharmacological alterations of DA levels significantly affect attentional accuracy in a rewarding attention task (Pezze et al., 2006). Furthermore, sexually motivated behaviour (Pfaus and Phillips, 1991) or lever pressing effort and approach behaviour for learned food rewards was also altered with pharmacological manipulations of DA levels in brain regions associated with reward (Ikemoto and Panksepp, 1996, Salamone and Correa, 2002). For example, administration of DA antagonists (e.g. pimozide) or depletion of DA suppressed the lever pressing response for natural rewards (e.g. food or water) in rats (Wise et al., 1978, Gerber et al., 1981) and decreased the rate of electrical brain self-stimulation (Cooper et al., 1978, Stellar and Corbett, 1989), whereas DA agonists (e.g. apomorphine) augmented the reward seeking behavior (Royall and Klemm, 1981). These observations suggest that DA signals could be important in attributing salience of reward and affecting the state of general alertness and motivation, a highly advantageous state for the successful pursuit of reward (Flagel et al., 2010).

The significance of reward learning and incentive salience hypotheses is particularly evident in response to drug cues and their ability to drive attention, refresh the memory of the rewarding experience and elicit a motivational state that can lead to approach behavior. Laboratory studies have demonstrated that cues associated with drugs (e.g. cocaine, nicotine, alcohol) can elicit drug-seeking behaviour in animals (Stewart et al., 1984, Katner et al., 1999, Weiss et al., 2001, Liu et al., 2006). In humans, both

clinical observations and laboratory studies indicate that following repeated pairing with drugs of abuse with cues, drug cues can cause attentional biases (Cox et al., 2006, Hogarth et al., 2008, Field et al., 2009) and acquire incentive motivational rewarding properties (Foltin and Haney, 2000, Panlilio et al., 2004, Childs and de Wit, 2009, 2011). These drug cues have been reported to function as conditioned reinforcers (Foltin and Haney, 2000, Kearns et al., 2005), increase drug seeking (Panlilio et al., 2004, Hogarth et al., 2007), and drug self-administration (Droungas et al., 1995, Mucha et al., 1998, Hogarth et al., 2010). These findings suggest that cue-induced drug-seeking behavior is strongly influenced by DA transmission. Hence establishing an understanding of the mechanisms and pathways through which this signal is regulated appears to be of paramount importance.

In summary, accumulating evidence suggests that DA influences the ability of incentive stimuli to elicit and sustain approach and investigation, facilitating the learning of associations between events. Once DA has imbued a stimulus with incentive salience, that stimulus itself can come to be rewarding, and reinforce other behaviors and maintain effortful goal-seeking.

Cue-induced DA response in mesocorticolimbic pathways

DA's function in regulating cue-reward learning and behavior varies in mesocorticolimbic regions. In the striatal regions, DA signalling in response to cues has been correlated with the development of flexible goal-directed behaviors, reward-cue associations, and stimulus-response habits. Although the role of striatal regions and the contribution of DA signalling in mediating drug cue-response is relatively well established, the function of extra-striatal regions in regulating this effect remains largely unclear. The animal literature shows that extra-striatal regions such as the anterior cingulate, OFC, amygdala and hippocampus can directly and indirectly alter DA signalling in the striatum (Karreman and Moghaddam, 1996, Floresco et al., 1998, Floresco et al., 2001, Goldstein et al., 2007). Furthermore, disruption of activity in these regions (by pharmacological inactivation or lesioning) can disrupt reinstatement behavior in response to drug cues, a behaviour associated with neurotransmission of DA (McLaughlin and See, 2003). In humans, a rapidly growing body of functional

neuroimaging studies has characterized a network of brain regions that are associated with cue reactivity. The amygdala, anterior cingulate, OFC and hippocampus are the most consistently addressed regions (Chase et al., 2011, Tang et al., 2012). The mentioned brain regions create circuits that are highly interactive and communicate through transmission of a variety of neurotransmitters such as DA, glutamate and GABA (Uys and Reissner, 2011, Hearing et al., 2012). However, considering all we have discussed so far, DA is proposed to be the signal mostly implicated in regulating these pathways (Blum et al., 2012). Although the study of neurotransmitter signalling and the role of DA in particular was previously not possible for some brain regions and circuits that are implicated in addiction, recent methodological advances have fortunately provided the opportunity to directly test this proposition in humans. Our study benefits from these methods.

Among the extra-striatal regions, the amygdala and hippocampus are highlighted in both animal and human studies for being particularly important in cue-induced responses.

Amygdala and cues

Recent evidence indicates that among the limbic regions, the amygdala has a particularly significant role in regulating the behavioral response to drug cues. First, lesioning and pharmacological inactivation of the amygdala abolishes cue-induced cocaine reinstatement in rats (Meil and See, 1997, Kantak et al., 2002). Second, electrophysiological studies demonstrated that stimulation of the basolateral nucleus of the amygdala (BLA) led to reinstatement of cocaine seeking after extinction (Hayes et al., 2003) and neuronal firing was augmented during exposure to cocaine cues (Carelli et al., 2003). Third, at a cellular level, exposure to cocaine cues increased fos protein expression - a measure of neuronal changes- in motivational learning in the BLA (Neisewander et al., 2000). Moreover, stimulation or inhibition of synaptic transmission through signal regulated kinases in the central amygdala (extracellular signal-regulated kinase; ERK) respectively augments or suppresses cue-induced cocaine seeking (Lu et al., 2006). Interestingly, lesions of the BLA do not impair cocaine primed reinstatement (McFarland and Kalivas, 2001). This observation indicates that the specific role of the amygdala is

probably not associated with reinforcement of the reward itself but rather allocating incentive salience to the reward predicting stimuli. Studies assessing the role of the amygdala in drug cue activation in humans are few (Chase et al., 2011, Tang et al., 2012). Several functional imaging studies have reported increased glucose metabolism, cerebral blood flow and fMRI BOLD signal in the amygdala when drug dependent users were presented with cocaine cues (Grant et al., 1996, Childress et al., 1999, Bonson et al., 2002). The observed metabolic and neuronal activity in the amygdala during drug cue presentation implies that the amygdala plays an active role in regulation of stimulus-response association. These findings are in accordance with the body of literature that supports the amygdala's role in memory and reward processes, including acquisition and learning of cue-reward association, consolidation of corresponding memories and expression of reward-seeking behavior (Grimm and See, 2000).

Although the amygdala's role in regulating the response to both natural and drug stimuli is supported by the literature, the neurochemical correlates of this activity are not clearly defined. Considering the previously discussed role of DA in motivation, learning and cue reward association, this neurotransmitter seems a plausible candidate. In support of this claim, Weiss et al. reported that DA transmission is enhanced in the NAcc when rats are exposed to cocaine cues (Weiss et al., 2000). Furthermore, several studies have demonstrated that pharmacologic manipulations of DA levels in the amygdala influence the behavioral response to cocaine cues (Alleweireldt et al., 2002, Di Ciano et al., 2003, Berglind et al., 2006) and affect learning and memory of the cue-drug association (Hitchcott et al., 1997). These laboratory findings support the role of the DA signal in cue-induced activity in the amygdala. However, due to the methodological limitations of imaging studies the study of neurotransmitters, specifically that of DA, in regulation of amygdala activity is lacking in humans. Fortunately, recent advances in neuroimaging techniques have now made it possible to begin studies in humans. For example the newly developed tracer fallypride can detect DA signaling in extra-striatal regions such as the amygdala.

Hippocampus and cues

The hippocampus might also influence responses to reward cues including those associated with abused drugs (Shohamy and Adcock, 2010). For example, several studies have shown that electrical stimulation of the hippocampus can elicit drug-seeking behavior in rats (Vorel et al., 2001, Taepavarapruk and Phillips, 2003) while inactivation of the ventral subiculum can decrease cue induced reinstatement (Sun and Rebec, 2003) (Rogers and See, 2007). In humans, functional neuroimaging studies have identified drug cue-induced activations of the hippocampus (Grant et al., 1996). The exact role of hippocampal activations in cue related relapse, though, remains to be defined.

Our understanding of the specific functions of the neurotransmitters involved in regulating reward-related activity of the hippocampus is still preliminary. However, the following observations suggest that DA is a contributing factor: (i) DA transmission elsewhere influences reward associated behaviors, (ii) mesolimbic DA cells project to the hippocampus (Gasbarri et al., 1994, Shohamy and Adcock, 2010), (iii) DA receptors are present in the hippocampus (Goldsmith and Joyce, 1994), and (iv) stimulant drug administration (cocaine and amphetamine) increases extracellular DA levels in the hippocampus (Borgkvist et al., 2012). Some PET studies have reported displacement of [^{18}F]fallypride in the hippocampus after the injection of amphetamine in primates (Nagano et al., 2000, Slifstein et al., 2004).

The functional significance of this DA signaling in the hippocampus is still not fully understood. Recent evidence, though, supports a role for hippocampal DA in the induction of experience-dependent neuroplastic changes, possibly including aspects of memory (Frey et al., 1990, Otmakhova and Lisman, 1998). For example, laboratory studies indicate that DA transmission enhances LTP in CA1 hippocampal pyramidal cells in vitro (Li et al., 2003). In humans, fMRI studies have reported an association between the activity in the hippocampus and midbrain DA areas during memory and learning tasks (Wittmann et al., 2005, Adcock et al., 2006). Studies to directly investigate cue induced DA release in the hippocampus are few. However, two recent reports suggest that blocking the D1 and D2 receptors in the dorsal hippocampus decreases intra-VTA morphine-induced conditioned place preference (CPP) (Esmaeili et al., 2012) while administration of methamphetamine in the ventral hippocampus produced positive place

reinforcement learning 24 h following conditioning. (Keleta and Martinez, 2012). Taken together, these findings implicate the hippocampal DA transmission in the regulation of response to reward related cues including those associated with addictive drugs. In the present Master's thesis, I used high-resolution PET imaging with [^{18}F]fallypride to test whether drug related cues could induce evidence of DA release in the amygdala and hippocampus in humans with a history of cocaine dependence.

Specific Goals and Hypothesis of the Thesis

Mesocorticolimbic DA transmission signals the availability of a desirable reward leading to sustained interest in reward paired cues. Cue-induced reinstatement is proposed to involved DA signalling in these pathways that include the striatal and limbic regions (such as amygdala and hippocampus). This project proposes to explicitly test the effect of drug cues on DA release within the striatum and extra-striatal regions implicated in reward related behaviors and drug relapse.

The primary objectives are:

1) Cocaine cues will increase extracellular DA levels in striatum, amygdala and hippocampus.
2) Brain regional individual differences in DA responses will predict drug craving.

Cocaine Cue-Induced Dopamine Release in Amygdala and Hippocampus: A High-Resolution PET [^{18}F]Fallypride Study in Cocaine Dependent Participants

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ABSTRACT

Background: Drug related cues are potent triggers for relapse in people with cocaine dependence. Dopamine release within a limbic network of striatum, amygdala and hippocampus has been implicated in animal studies, but in humans it has been possible to measure effects in the striatum only. The objective here was to measure dopamine release in the amygdala and hippocampus using high-resolution PET with [^{18}F]fallypride.

Methods: Twelve cocaine dependent volunteers (mean age: 39.6±8.0; years of cocaine use: 15.9±7.4) underwent two [^{18}F]fallypride HRRT PET scans, one with exposure to neutral cues and one with cocaine cues. [^{18}F]Fallypride non-displaceable binding potential (BP_{ND}) values were derived for five regions of interest (ROI) (amygdala, hippocampus, ventral limbic striatum, associative striatum, and sensorimotor striatum,). Subjective responses to the cues were measured with visual analog scales and grouped using principal component analysis.

Results: Drug cue exposure significantly decreased BP_{ND} values in all five ROI in subjects who had a high but not low craving response (limbic striatum: p=0.019, associative striatum: p=0.008, sensorimotor striatum: p=0.004, amygdala: p=0.040, and right hippocampus: p=0.025). Individual differences in the cue-induced craving response predicted the magnitude of [^{18}F]fallypride responses within the striatum (ventral limbic: r=0.581, p=0.048; associative: r=0.589, p=0.044; sensorimotor: r=0.675, p=0.016).

Conclusions: To our knowledge this study provides the first evidence of drug cue-induced dopamine release in the amygdala and hippocampus in humans. The preferential induction of dopamine release among high craving responders suggests that these aspects of the limbic reward network might contribute to drug seeking behavior.

INTRODUCTION

The amygdala and hippocampus potently influence learning and memory (Robbins et al., 2008), responses to motivationally important cues (Tracy et al., 2001, Tye and Janak, 2007), and the development and expression of habit-like behaviors (Lingawi and Balleine, 2012). Less attention has been given to how they affect responses to drug related cues, but lesioning or inactivating these regions diminishes cue precipitated drug-seeking behaviors (Meil and See, 1997, Kantak et al., 2002, Rogers and See, 2007) whereas electrical stimulation increases them (Vorel et al., 2001, Hayes et al., 2003). In humans, functional neuroimaging studies have identified both amygdalar and hippocampal activations to drug related cues (Grant et al., 1996, Childress et al., 1999, Wexler et al., 2001), but the neurotransmitters mediating these effects remain unknown.

One plausible candidate transmitter is dopamine (DA). Mesolimbic DA transmission is thought to influence the ability of drug cues to capture and sustain interest, and foster the development and expression of habit-like, stimulus-response behaviors (Berridge, 2007). In laboratory animals, these effects have been studied primarily within the striatum. However, exposure to cocaine cues can also induce DA release within the amygdala (Weiss et al., 2000), an effect known to influence cue-induced cocaine seeking behavior (See et al., 2001, Ledford et al., 2003, Berglind et al., 2006). The role of hippocampal DA transmission on responses to drug cues remains unknown, but emerging evidence supports an influence in the formation and activation of emotionally potent memories (Shohamy and Adcock, 2010). Together, these observations highlight the importance of DA transmission within multiple regions in the acquisition, selection and maintenance of reward seeking behaviors.

In humans, drug cue-induced DA responses have been reported in the striatum (Volkow et al., 2006, Wong et al., 2006, Boileau et al., 2007) but not elsewhere in the brain reflecting limitations of the PET tracer, [^{11}C]raclopride. A more recently developed tracer, though, [^{18}F]fallypride, has higher affinity than [^{11}C]raclopride for D2/D3 receptors enabling the measurement of DA release in regions where the concentration of DA receptors is substantially lower than striatum (Mukherjee et al., 2002, Slifstein et al., 2010). In the present study, we used this tracer with high-resolution PET to measure the

ability of drug cues to induce DA release in the amygdala, hippocampus and striatum of volunteers meeting diagnostic criteria for cocaine dependence.

METHODS and MATERIALS

Participants

Non-treatment seeking cocaine users who met DSM-IV criteria (American Psychiatric association,2000) for current Cocaine Dependence were recruited from the community through local advertisements. Volunteers who tentatively met the entry criteria following a brief telephone screen were invited to a more in-depth face-to-face evaluation using the Structured Clinical Interview for DSM-IV (First, 1997). Participants were free of current axis I psychiatric disorders other than substance use, had never experienced head trauma with loss of consciousness, and were physically healthy as determined by a medical exam, electrocardiogram and standard laboratory tests. Women were excluded if they had a seropositive pregnancy test. All participants had a current or past history of other illicit substance use but reported cocaine as their drug of choice (Supplement Table 1). No participants were currently seeking treatment for their substance use problems or planning to quit in the next month. The study was carried out in accordance with the Declaration of Helsinki and approved by the Research Ethics Board of the Montreal Neurological Institute. All participants provided written, informed consent.

Procedure

Each subject had one MRI and two PET sessions carried out on separate days. Subjects were asked to abstain from psychotropic drugs for at least 24 hours before the PET sessions, and on the morning of each test day, urine drug screens were administered (Triage Drugs of Abuse Panel, Biosite Diagnostics, sensitive to amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, and phencyclidine) and results were recorded. Female participants were tested during the follicular phase of the menstrual cycle, and given a urine pregnancy test prior to each PET session; none tested positive (Assure FastRead hCG Cassette, Conception Technologies, San Diego, California).

On the neutral cue session (Figure 1), participants developed, two hours before scanning began, an autobiographical script with the investigator in which they recalled a relaxing, uneventful day that they could clearly remember and narrate in detail. The development and rehearsal of this script lasted 30 minutes. They were then presented with paperclips, pencils and erasers, asked to doodle or write a few sentences and erase them, and manipulate the paperclips. This object manipulation lasted about 15 minutes. Subjects were then shown a 10-minute video clip of people in everyday situations. Additional non-drug themed neutral videos were watched while lying on the PET bed.

Procedures were similar on the cocaine cue test session (Figure 1). Two hours before scanning began participants developed an autobiographical script with the investigator in which they described in detail a positive drug experience. Intranasal cocaine powder users were presented with a mirror, a razor blade, a straw, and a bag of white powder (lactose). Crack cocaine users were provided with a crack pipe, a spoon and a stone shaped crystal (salt). Subjects were told that the substance was genuinely cocaine or crack. Subjects were asked to use the razor to divide the powder into lines several times and to hold the straw, or touch and smell the crystal and put it in the pipe or spoon. This object manipulation lasted 15 minutes. For the following 15 minutes subjects watched a cocaine themed video. Additional cocaine themed videos were watched while lying on the PET bed. The videos showed images of people buying, using, and becoming intoxicated by cocaine (powder or crack depending on the subject's preferred form of the drug), as well as images of the drug itself and drug paraphernalia.

Neuroimaging

Each participant underwent two PET scans on a Siemens high resolution research tomograph (HRRT) and one T_1-weighted MRI session for PET/MR co-registration. PET sessions consisted of a bolus injection of 3.30±2.34 mCi [^{18}F]fallypride and two dynamic image acquisition scans (90-min and 60-min) separated by a 30-min break. A 6-min ^{137}Cs transmission scan for attenuation correction was performed at the beginning and end of every scan session.

[^{18}F]Fallypride non-displaceable binding potential values ($BP_{ND} = F_{ND} * (B_{avail} / K_D)$) were calculated (Cunningham et al., 1991, Innis et al., 2007) using the Simplified

Reference Tissue Model (SRTM) (Lammertsma and Hume, 1996) with the basis functions method (Gunn et al., 1997). The gray matter of the cerebellum was used as the reference region as it is devoid of D2/D3 receptors. Regions of interest (ROIs) were defined on each individual's MRI in stereotaxic space, and BP_{ND} values were derived for inter-group comparisons using Turku PET centre tools (www.turkupetcentre.net/). Regional BP_{ND} values were weighted with the volume size when combining both hemispheres (See Supplemental section for additional details).

Regions of Interest Analysis

We focused on a restricted number of *a priori* defined ROI based on the areas implicated in cue responsivity and the ability of [^{18}F]fallypride to detect effects there. The striatal sub-regions were based on the functional organization of limbic, associative and sensorimotor sub-compartments as proposed by Laruelle, Haber and colleagues (Haber and McFarland, 1999, Mawlawi et al., 2001, Martinez et al., 2003): ventral striatum (limbic striatum), pre-commissural dorsal caudate (posterior caudate / associative striatum), pre-commissural dorsal putamen (posterior putamen / associative striatum), post-commissural caudate (anterior caudate / associative striatum), and post-commissural putamen (anterior putamen / sensorimotor putamen). The two extra-striatal regions were hippocampus and amygdala. Regions were segmented using F.I.R.S.T. (FMRIB's Integrated Registration and Segmentation Tool) (www.fmrib.ox.ac.uk/fsl/first/index.html) (Patenaude et al., 2011), and then checked and modified manually if necessary.

Behavioral Measures

Drug craving and subjective mood states were assessed using 17 Likert-like visual analog scale (VAS) items (*happy, rush, high, euphoria, excited, anxious, energetic, mind-racing, alert, bored, interested, urge for cocaine, desire cocaine, crave cocaine, want cigarette, want alcohol and want other drug*). The VAS questionnaire was administered at baseline, 30 minutes before the start of the scan and then every 30 minutes after the start of the scan. The Cocaine Selective Severity Assessment Scale was administered as a measure of early cocaine abstinence symptoms at the baseline of each scan day

(Kampman et al., 1998). The total score was used as a measure of subjective withdrawal state.

Statistical Analysis:

All data were analyzed using IBM® SPSS® Version 20 for Macintosh. Data were analyzed using the GLM procedure for repeated measures to model three within subject factors of Hemisphere (Left and Right), Region (limbic striatum, associative striatum, sensorimotor striatum, amygdala and hippocampus), and Session (Neutral, Cocaine Cue), and one between subjects factor of Group (High craving, Low craving). Mauchly's test of sphericity suggested that the GLM, including both striatal and extrastriatal regions, violated the assumption of homogeneity of variance. We corrected for this by using lower-bound estimates to assess significance in the ANOVA; this is the most conservative correction available. Reanalyzing the data as separate ANOVAs for striatal and extra-striatal regions avoided the homogeneity issue but increased the risk of Type I errors due to failure to correct for multiple testing. Since the results were consistent with both analyses, we included all ROIs in one ANOVA and chose the more conservative option (lower-bound estimates of sphericity). Planned pairwise comparisons were performed to delineate the source of significant differences on ANOVA.

To estimate cue-induced change in subjective states, an average change from baseline score was calculated for each individual in each test session (delta score) and compared with Student's paired t-test. Because of substantial colinearity of the VAS items, distinct factors were generated. In brief, differences in VAS delta scores between the two sessions were calculated. These double delta scores were then grouped using principal component analysis. Factors with eigenvalues above one were extracted and varimax rotated when more than one factor was detected.

Individual differences in the magnitude of regional BP changes ($\%\Delta BP_{ND} = (BP_{ND\_Neutral} - BP_{ND\_cue})/ BP_{ND\_Neutral} * 100$) were correlated with subjective states using Pearson product moment correlations. In all analyses statistical significance was set as $p \leq 0.05$. Data normality for BP change scores were assessed with the Shapiro-Wilk test and met the assumption of normality.

RESULTS

Characteristics of Participants

Twelve volunteers completed the study (Table 1). Participants reported smoking crack cocaine (N=9) or taking it intra-nasally (N=3) at least once a week for an average of 16 years (range: 3 – 25 years, average 7.5±4.5 grams of cocaine per week). All participants had a current or past history of other illicit substance use (Supplement Table S1) but reported cocaine as their drug of choice. No participants were currently seeking treatment for their substance use problems.

Table 1: Characteristics of research participants (N=12).

Characteristics	Value (Mean ± SD)
Age (years)	39.5 ± 8.0 (range, 31 to 48 y)
Sex (number)	male (10/12)
Ethnicity	3 African Americans, 1 Aboriginal, 8 Europeans
Age of first use (years) [a]	23.7 ± 6.5
Duration of use (years)	15.9 ± 7.4 (range3 to 25 y)
Lifetime use (days)	2100.3 ± 1548.5
Cocaine use days / week, past 5 years[b]	4.3 ± 2.1
Amount / week (g)	7.5 ± 4.5
Primary route of administration	9 smoked cocaine, 3 intranasal powder
Cigarette smokers	9 current smokers

[a] *Drug use information refers to cocaine use and was collected through a self-report retrospective interview.*
[b] *For subjects with less than 5 years history of use, this number equals lifetime use.*

Subjective States Analysis

Exposure to the cocaine cues, as compared to the neutral cues, significantly increased drug craving scores (urge, desire, crave cocaine), effects that were maintained throughout the PET scanning session (p-values <0.005) (Figure 2). Cocaine cue exposure also increased scores for *Rush, Anxious, Excited, Mind-racing, Interested and Euphoria* (all $t_{(11)} > 2$, $p<0.04$); however, since many of the VAS measures were highly inter-correlated, reflecting a smaller number of latent constructs, principal component analysis was used to extract factors from the time averaged double delta VAS scores. Six distinct

factors were identified (Supplement Table 2). The first factor accounted for 31% of the variance and included four items: crave cocaine (0.94), desire cocaine (0.85), urge for cocaine (0.85) and alert (0.75). This factor appeared to represent focused craving for cocaine; it was used in the subsequent correlational analysis and to divide subjects into those who did (n=6) vs. did not (n=6) report positive changes in the crave factor score.

PET [^{18}F]Fallyride BP_{ND} Data: Effect of Cocaine Cues

The four-way Group x Session x ROI x Hemisphere ANOVA of BP_{ND} values yielded a three-way Group x Session x ROI interaction ($F_{1,10}$= 9.02, p=0.013). Decomposition of the interaction indicated that this reflected significant cue-induced decreases in [^{18}F]fallypide BP_{ND} among the high craving subjects (Figure 3; Supplement Table 3a , Supplement Figure 1). Among subjects exhibiting high craving factor scores, exposure to the cocaine cues, compared to the neutral ones, led to significantly lower BP_{ND} values in the limbic p=0.019, associative p=0.008, and sensorimotor p=0.004 striatum, as well as the amygdala (p=0.040). Significant effects were not seen in the whole hippocampus. However, further exploration suggested an effect in the right hippocampus (ANOVA posthoc: p=0.047; t-test: $t_{(5)}$=3.15, p=0.025). These effects were not seen in subjects with low craving scores (p≥0.1) (Figure 3; Supplement Table 3b).

As found in two PET [^{11}C]raclopride studies (Volkow et al., 2006, Wong et al., 2006) individual differences in cocaine cue-induced craving predicted differences in the measure of striatal DA release. The greater the craving response, the greater the DA response. This association was observed in the striatum as a whole (r=0.631, p=0.028) and in all three striatal ROIs: limbic (r=0.581, p=0.048), associative (r=0.589, p=0.044), and sensorimotor (r=0.675, p=0.016) (Figure 4). The effects were in the same direction when hemispheres were investigated independently and reached significance in left associative (r=0.604, p=0.038), left sensorimotor (r=0.773, p=0.003) and right limbic striatum (r=0.626, p=0.029). Correlations between craving and changes in [^{18}F]fallypride BP_{ND} were not significant in the hippocampus (r=0.213, p=0.51) or amygdala (r=0.258, p=0.42).

DISCUSSION

To our knowledge the present study provides the first evidence of drug cue-induced DA release in human amygdala and hippocampus. The amygdala is thought to play an important role in the acquisition and expression of learned associations between emotionally important events. In conjunction with activity in the striatum and hippocampus, these effects influence the ability of motivationally salient stimuli to elicit and sustain focused interest and facilitate the selection of situation appropriate behavioral responses (Robbins and Everitt, 2002, Phillips et al., 2003, Goto and Grace, 2008, Robbins et al., 2008, Shohamy and Adcock, 2010).

In humans, the role of the amygdala in the processing of emotionally relevant stimuli has been studied using various methods, including functional neuroimaging (Chase et al., 2011, Tang et al., 2012), assessments of the effects of naturally occurring selective lesions (Adolphs et al 1995; Tsuchiya et al 2009), and following direct electrical stimulation (Rayport et al 2006). Together, these studies are consistent with a more extensive animal literature indicating that the amygdala can modulate associative learning between discrete cues and rewards, influence the emotional intensity attached to events, and regulate striatal responsiveness and its effects on behavioral approach (Savage and Ramos, 2009, Buffalari and See, 2010). Although few studies have investigated which specific neurotransmitters are implicated, DA is a plausible candidate. For example, in laboratory animals, exposure to cocaine cues increases DA release in the amygdala (Weiss et al., 2000). Moreover, several studies have demonstrated that pharmacological manipulations of DA levels in the amygdala influence the behavioral response to cocaine cues (Alleweireldt et al., 2002, Di Ciano et al., 2003, Berglind et al., 2006) and affect learning and memory of the cue-drug association (Hitchcott et al., 1997). Our own study raises the possibility that cue-induced amygdalar DA release plays a similar role in humans.

To the best of our knowledge this is also the first report of a dopaminergic response to cocaine cues in the hippocampus in both the animal and human literatures. A role of the hippocampus in episodic memory, reward learning, and the generation of contextually appropriate reward seeking has been indicated, though (McDonald & White

1993, Eichenbaum, 2013, Dickerson and Eichenbaum, 2009). Several studies have demonstrated that DA transmission facilitates hippocampal synaptic long-term potentiation (LTP) (Jay, 2003, Li et al., 2003) and likely has an important role in the formation and reactivation of reward related memories (Shohamy and Adcock, 2010, Frey et al., 1990, Otmakhova and Lisman, 1998). In humans, neuroimaging studies have provided evidence of hippocampal activation following exposure to drug cues (Grant et al., 1996, Kilts et al., 2001, Wexler et al., 2001 Chase et al., 2011, Tang et al., 2012). Moreover, activity in dopaminergic midbrain regions evoked by reward anticipation tasks is associated with hippocampal activation and evidence of enhanced hippocampus-dependent long-term memory formation (Wittmann et al., 2005, Adcock et al., 2006). Thus the hippocampal DA signal may influence neuroplastic changes that facilitate long-term memories of pairings between rewards and context.

In the present study, cue-induced DA release was also observed in the striatum. Evidence of cocaine cue-induced striatal DA responses has been seen previously in PET studies with [^{11}C]raclopride (Volkow et al., 2006, Wong et al., 2006). As observed here, individual differences in the magnitude of the striatal DA effect co-varied with self-reported craving. Based on studies conducted in laboratory animals, it has been proposed that cue-induced DA release within the ventral striatum facilitates flexible, goal-directed approach toward reward-related stimuli (Weiss et al., 2000, Nicola et al., 2005, Berridge, 2007). DA release in more dorsal regions of the striatum, in comparison, may more closely reflect the acquisition and promotion of habit-like, stimulus-response behaviors (McDonald and White, 1993, Ito et al., 2002, Vanderschuren et al., 2005). Accumulating evidence, though, suggests that the primate striatum is not parcellated into sharply delineated subregions; rather there is a gradation of limbic cortical input, innervating ventromedial aspects most densely, dorsolateral aspects least so. Whereas the ventral striatum receives dense input from the amygdala, hippocampus and limbic cortex, more dorsal aspects receive more input from associative and sensorimotor cortex (Haber and Knutson, 2010).

The midbrain DA system includes projections from the substantia nigra to dorsal striatum and more limbic directed projections from the ventral tegmental area to the nucleus accumbens, basolateral and central nuclei of the medial amygdala, and

hippocampus; DAergic innervation of the latter structure is more dense in primates than in rodents (Haber and Knutson, 2010). As noted above, reciprocal innervation is evident also, and stimulating the afferent fibers from the amygdala and hippocampus increases accumbal DA release (Floresco et al., 1998, Floresco et al., 2001). Our finding of DA responses to cocaine cues in all three regions – amygdala, hippocampus and striatum – supports the view of limbic and striatal structures as components of an integrated system, contributing to the incentive salience of motivationally relevant cues (Robbins and Everitt, 2002, Phillips et al., 2003, Goto and Grace, 2008, Shohamy and Adcock, 2010).

The observation that cue-induced DA responses occurred only in the high craving subgroup may reflect a number of factors. First, the videos contained narrative detail designed for the local milieu, and the autobiographical script would be expected to enhance these effects, but some participants might be non-responsive to the mostly impersonal cues (O'Brien et al., 1979, Staiger and White, 1991, Conklin et al., 2010). Alternatively, our low craving participants may have had less intent to use drugs that day; active inhibition of craving can affect cue-induced appetitive states and cortico-limbic activity (Wertz and Sayette, 2001, McBride et al., 2006, Volkow et al., 2010, Prisciandaro et al., 2012). Finally, recent animal studies suggest that DA responses to reward related cues occur only in those subjects that imbue the cues with incentive salience; individual differences in these tendencies appear to be an inherited trait (Robinson and Flagel, 2009, Flagel et al., 2010). The higher craving individuals in our study might be particularly prone to attribute incentive salience to drug cues. Intriguingly though since both sub-groups had extensive cocaine use histories, the observations might identify two separate neurobiological pathways to addiction.

Our findings should be interpreted in light of the following considerations. First, consistent with two PET [^{11}C]raclopride studies in cocaine dependent participants (Volkow et al., 2006, Wong et al., 2006), we observed evidence of cue-induced DA responses in the dorsal striatum. In comparison, in healthy volunteers administered only three doses of *d*-amphetamine, exposure to drug-paired cues led to DA release in the ventral striatum (Boileau et al., 2007). In the present study, cue-induced DA responses were seen in both the dorsal and ventral striatum. This more widespread effect could reflect the presence of relatively more diverse cues (*e.g.,* videos, autobiographical

memories and paraphernalia), the fact that our participants were not inpatients but free to depart after the test sessions and potentially use cocaine, or the use of a different tracer plus higher resolution camera. These features noted, the statistically most robust effect was seen in sensorimotor striatum which overlaps with the area preferentially activated in the [^{11}C]raclopride studies (Volkow et al., 2006, Wong et al., 2006). Moreover, the present results suggest that exposure to a mix of personalized and novel cues evocative of highly learned reward related memories and behaviors can lead to activations of both ventral and dorsal aspects of the striatum. Second, in our study, individual differences in craving did not correlate with the magnitude of DA response in the amygdala and hippocampus. One possibility is that, compared to the striatum, dopamine responses in these regions are somewhat less closely related to the initiation of approach behaviors, and more closely related to stimulus intensity, context and associative learning (Everitt & Robbins 2005). Third, our PET scans were three hours in duration (time post-tracer injection). There is broad consensus that 60 to 90 minutes is sufficient to detect effects outside of the basal ganglia (*i.e.,* amygdala and hippocampus); within the striatum, longer scans are required due to the greater time needed for fallypride to reach steady state levels. Simulation experiments suggest that a striatal signal begins to emerge after two hours (Ceccarini et al., 2012); empirical data indicate that tracer equilibrium is clearly achieved by three hours (Vernaleken et al., 2011). The present study plus work conducted elsewhere further confirm that scans of 180 to 210 minutes are sufficient to measure striatal DA release (Buckholtz et al., 2010a, Buckholtz et al., 2010b, Treadway et al., 2012). Fourth, the associations between cue-induced DA release and self-reported drug craving are correlations and do not indicate causality. However, other evidence indicates that DA contributes to susceptibility to craving states; *e.g.,* diminishing cocaine cue-induced increases in DA transmission leads to decreases in craving (Berger et al., 1996, Leyton et al., 2005). Fifth, the order of scans was fixed (neutral day first, followed by cocaine day) to avoid pairing the PET environment with drug cues prior to the neutral test session. This benefit of the design was considered reasonable since [^{18}F]fallypride binding exhibits good test-retest reliability (Mukherjee et al., 2002); indeed, if the effect seen here was due to the order of scan, it would not have been observed only in those

subjects reporting high levels of craving. Finally, our study had a small number of female participants. Future studies will be needed to address possible effects of gender.

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The authors have no competing financial interests to declare.

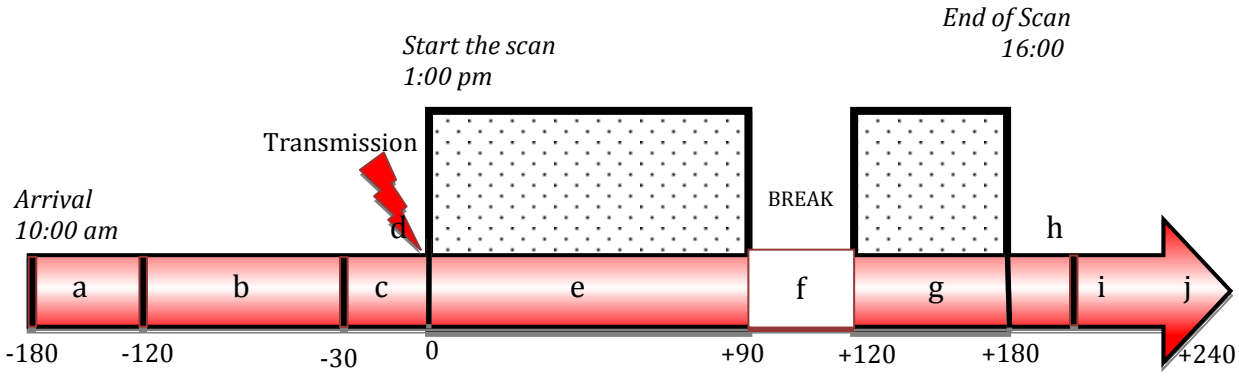

Figure 1: Test day procedures and timing. Time points are defined according to start of emission scan (time point 0). a. Arrival at the PET unit, baseline measurements and urine drug test. b. Develop autobiographical script, manipulate paraphernalia, watch video highlights (context different on neutral and cue day as described in supplement section in full detail). c. Collect subjective measures, lay down in camera, insert intravenous catheter for tracer injection. d. Six-min transmission scan e. Emission scan, watching videos through video glasses. f. 30-min break. g. Reinstall in the scanner, continue neutral or cue videos. h. Six-min transmission scan. i. End of the scan, removal from the scanner, self report of subjective measures. j. Debriefing.

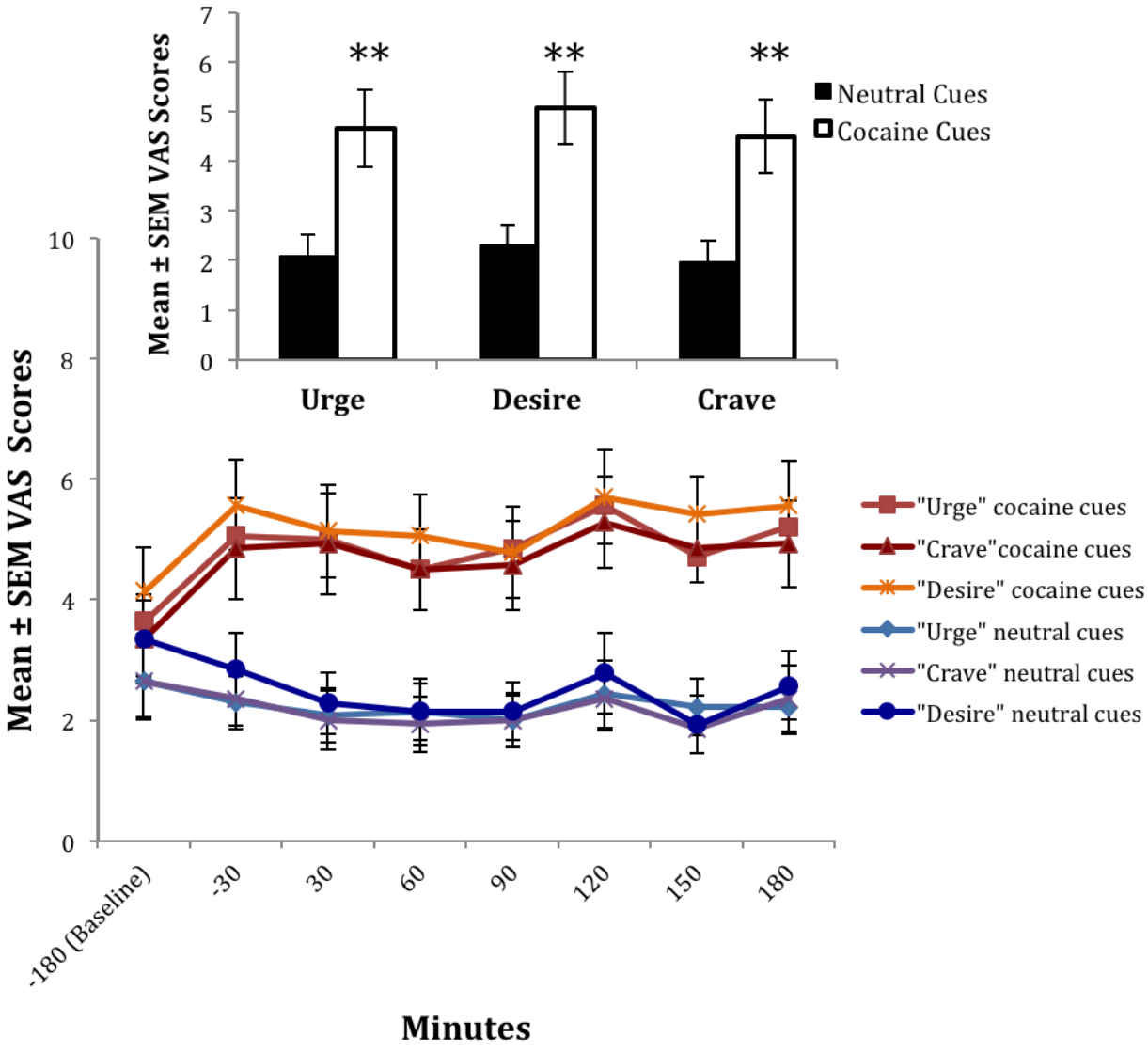

Figure 2: Changes of mean craving scores of self-report visual analog scales on neutral and cocaine cue day. Time points are defined according to start of emission scan (time point 0). Cue exposure starts approximately one hour after baseline time-point. Error bars represent standard errors of the mean (All t values>3.5, df=11, **p values<0.005).

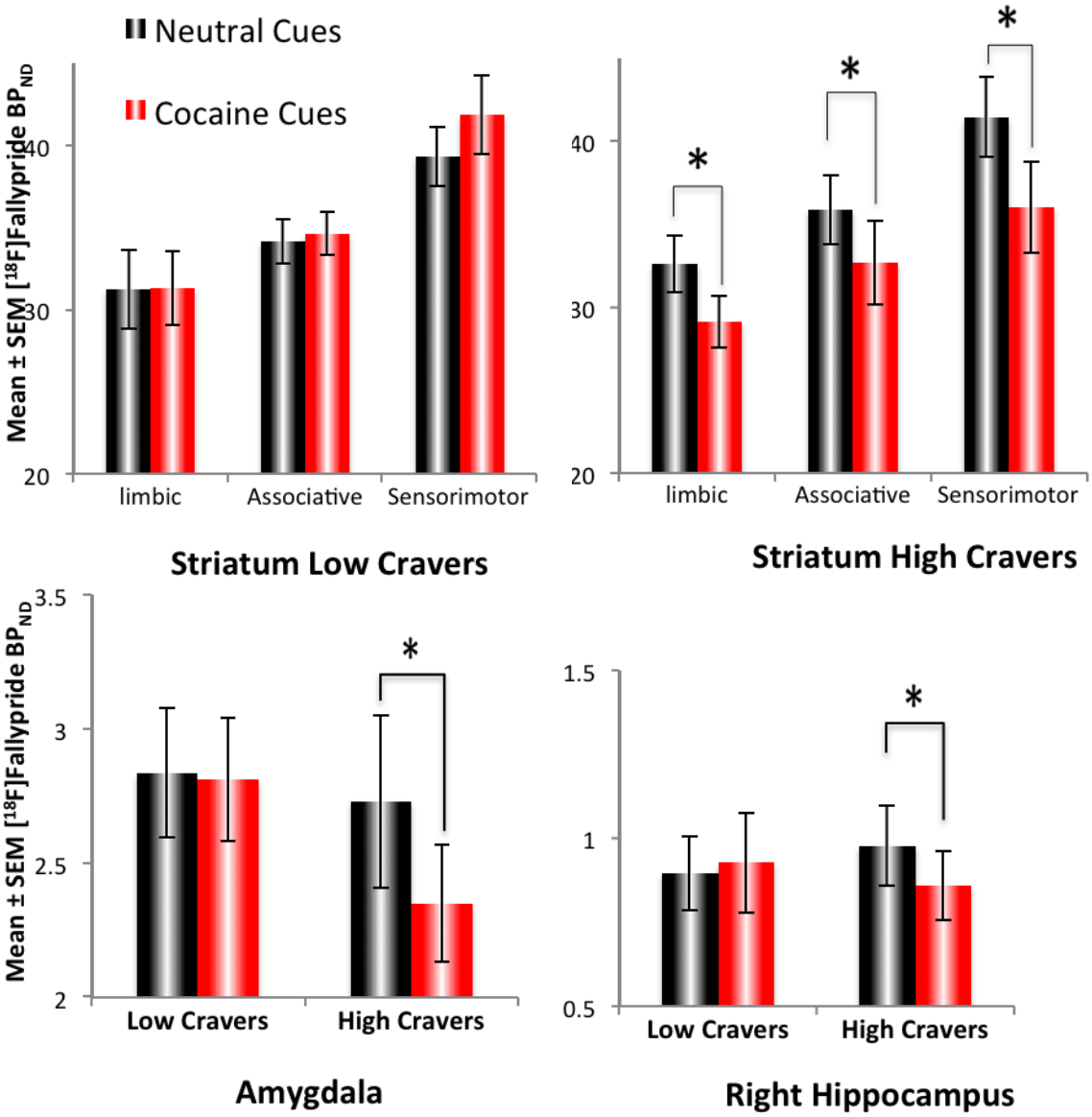

Figure 3: [^{18}F]Fallypride BP_{ND} on neutral and cocaine cue days in subjects with high and low craving factor scores
Top: Striatal regions. Bottom left: Amygdala. Bottom right: Hippocampus. Values represent mean ± SEM. * $p<0.05$

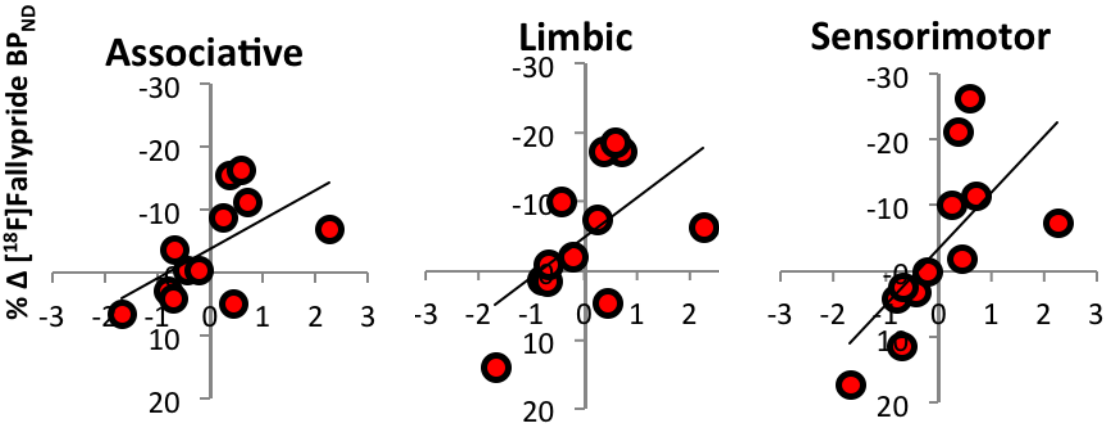

Figure 4: The relationship between changes in cue induced craving factor score (x axis) and percent changes in [^{18}F]fallypride BP_{ND} (y axis) across the test sessions in striatal ROIs (N=12). Associative striatum (r=0.589, p=0.044), ventral limbic striatum (r=0.581, p=0.048), and sensorimotor striatum (r=0.675, p=0.016).

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Supplement Section

Supplemental Methods

Procedure

Participants arrived at the Montreal Neurological Institute (MNI) at approximately 10:00am on each PET scan day. Collection of baseline subjective and physiological measurements and drug screen tests lasted approximately one hour. Participants then completed the neutral and cocaine cue sessions as described below. Heart rate and blood pressure were collected throughout. Following the cocaine cue session, subjects were seen by a psychiatrist and debriefed before being released.

Neuroimaging

The PET acquisitions were performed on a Siemens high-resolution research tomograph (HRRT) with the 3D acquisition mode yielding 207 transverse planes (voxel size = 1.2 cubic mm). Compared to its predecessor, the ECAT EXACT HR+ (CTI/Siemens), the HRRT provides considerable improvement in detection efficiency, sensitivity and spatial resolution (van Velden et al., 2009).

PET sessions consisted of a bolus injection of [^{18}F]fallypride and two dynamic image acquisition scans (90-min and 60-min), separated by 30-min break when subjects were allowed to leave the scanner. A 6-min ^{137}Cs transmission scan for attenuation correction was performed prior to every emission scan session. The 33 frames of dynamic PET images were reconstructed in time frames of progressively longer duration, from 10 to 600 seconds, including the break (30 minutes) at the 27th frame.

For all scans and for all conditions (scanning day 1 and day 2), the dose of radiotracer was in the range of 3.0 mCi to 3.8 mCi (mean±SD: 3.33±0.24 mCi for day 1; and 3.28±0.24 mCi for day 2). Furthermore, for all the scans the specific activity was in the range 900-1200 Ci/mmol. Taking the mean injected dose for each day, this corresponds to a mass dose in the range of 1.01 μg to 1.35 μg for day 1 and 1.00 μg to 1.33 μg for day 2.

Reconstructions were performed using OP-OSEM (Ordinary Poisson Ordered Subset Expectation Maximization: 10 iterations, 16 subsets) (Comtat et al., 2004; Hong et

al 2007) including compensation for dead-time, detector non-uniformities, attenuation, scattered and random coincidences and motion. The reconstructed image frames were composed of 256×256×207 voxels (voxel side length = 1.21875 mm). Motion correction for repositioning errors and potential head movement during the scans was based on an image-based automated algorithm (Costes et al., 2009) that estimates rigid-body motion between the dynamic frames. Emission data were then re-reconstructed taking into account mismatch between the transmission scan and each individual emission frame. The emission images for the different frames were then realigned to a common head pose.

Each participant underwent T_1-weighted MRI imaging for the purpose of PET/MR co-registration. The MRIs were obtained on a Siemens 1.5 tesla scanner from a 3D fast field echo sequence with sagittal acquisition and 160 slices at 1mm isotropic resolution (TR = 9.7 ms, TE = 4 ms, flip angle = 12°, FOV = 250 and matrix 256 × 256). Each MR image was first pre-processed with CIVET pipeline (version 1.1.9) (wiki.bic.mni.mcgill.ca/index.php/CIVET) developed at the MNI for fully automated structural image analysis (Zijdenbos et al., 2002, Ad-Dab'bagh et al., 2006). The native MR volume was normalized for intensity, corrected for non-uniformity (Sled et al., 1998), and linearly and non-linearly transformed into standardized stereotaxic space using automated feature-matching (Collins et al., 1994) to the ICBM152 template. The MR image in stereotaxic space was discretely classified into white matter, gray matter and CSF (Zijdenbos et al., 1998), and was automatically segmented in main brain structures using a probabilistic atlas based approach (ANIMAL) (Collins and Evans, 1997).

The spatial rigid-body transformation between the summed PET volume and the native MR image was estimated with normalized mutual information, and was used to position the region of interest (ROI) masks into the native PET space. The resulting registration was visually checked for the whole brain and at the level of basal ganglia.

[^{18}F]Fallypride BP_{ND} values express the relation between the estimated concentration of available DA D2/D3 receptors (B_{avail}), the dissociation constant of the radiotracer from D2/D3 receptors (K_d), and the free fraction of non-specifically bound

tracer in the brain (F_{ND}). The gray matter of the cerebellum (defined by tissue classification and brain segmentation) was used as the reference region as it is devoid of D2/D3 receptors. [^{18}F]Fallypride binding potential values were used for regions of interest (ROI) analyses. The regional BP values were weighted with the ROI size when combining regions on right and left hemisphere due to the natural asymmetry in some brain regions, in particular in the basal ganglia.

Regions of Interest Analysis

The analysis began with the segmentation of subcortical areas with a non-linear registration approach. Time-activity curves (TACs) were extracted from the original (non-smoothed) dynamic PET image with full accounting for decay, deadtime, scatter, randoms, attenuation, detector normalization and head motion (Costes et al., 2009) by eroding the ROI masks in order to reduce partial volume effects on the PET image. Mean [^{18}F]fallypride BP_{ND} values were then estimated for each ROI.

In order to explore striatal regions, an atlas defining ventral striatum, caudate and putamen was developed on the high resolution ICBM template (Fonov et al., 2009). Then based on the functional organization of limbic, associative and sensorimotor sub-compartments these three regions were manually subdivided (Mawlawi et al., 2001) into 5 anatomical sub-regions including ventral striatum (VS), pre-commissural putamen (Pre-DPU), pre-commissural caudate, (Pre-DCA), and post-commissural putamen (Post-DPU) (Martinez et al., 2003). Limbic striatum consisted of the ventral striatum, sensorimotor striatum was centered in posterior putamen, and associative striatum included the anterior putamen and both pre and post commissural caudate (Martinez et al., 2003).

Two extra-striatal regions were selected *a priori,* hippocampus and amygdala. The regions were automatically segmented using F.I.R.S.T. (FMRIB's Integrated Registration and Segmentation Tool) (Patenaude et al., 2011), checked and modified manually if necessary.

Behavior & Psychophysiology

Lifetime Drug Use and Depression

During the initial screening interview, all subjects completed self-report measures of previous drug and alcohol use including the timeline follow-back questionnaire (TLFB) and mood (Beck Depression Inventory) (Beck et al., 1961).

Cardiovascular

Psychophysiological measurements (heart rate, blood pressure) were obtained continuously throughout the PET sessions using an automated sphygmometer.

Supplemental Results

Physiological States

No significant change was observed in heart rate ($t_{(11)}$= -2.03, p=0.068) or blood pressure across the test sessions ($t_{(11)}$<1.2, p>0.25).

Subject Characteristics

No significant correlation was found between crave factor score and age, CSSA (withdrawal score), amount of cocaine/week and BDI score (N=12).

The high *vs*. low craving groups did not significantly differ on abstinence related symptoms on either the neutral or cue day (assessed by CSSA score), hours since last use of cocaine, interval between the two scan days, age, or BDI score (p> 0.1).

PET [^{18}F]Fallyride BP_{ND} Data

The three-way repeated measures ANOVA of BP_{ND} values (Session × ROI × Hemisphere) in all participants (N=12) yielded significant main effects of ROI ($F_{1,11}$= 548.79, p < 0.0001) and hemisphere ($F_{1,11}$ = 15.47, p = 0.002). The high vs. low cravers, though, did not differ in BP_{ND} values on the neutral cue test session ($t_{(10)}$<1.0 for all ROI, p values>0.4).

Correlations between craving and changes in [^{18}F]fallypride BP_{ND} were not significant in the hippocampus (p=0.42) or amygdala (p=0.51). The five other subjective state factors, which accounted for smaller portions of the variance in VAS, did not predict group differences or correlate with DA release in any of the ROI (Supplement Table 3)

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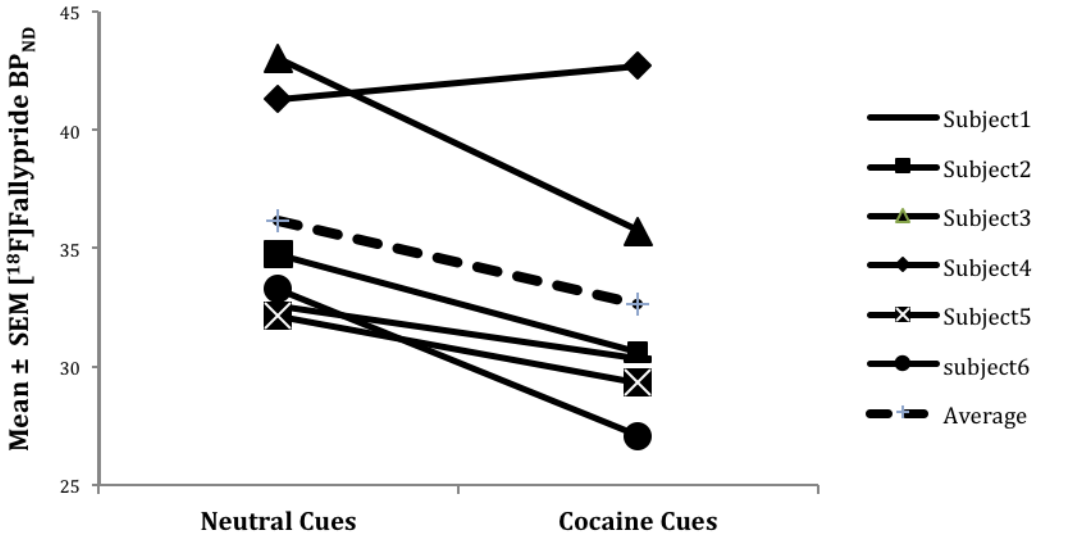
Supplemental Figures and Tables



Supplement Figure1: [^{18}F]fallypride BP_{ND} values in the whole striatum on neutral and drug cue day in high craving individuals. Dashed line represents the mean of the group.

Supplement Table 1: Self-Reported Drug Use			
Drug Class	**Mean**	**St. Dev**	**Number of users**
Alcohol (intoxication)			
Age of First Use	14.7	2.99	12
Lifetime days of use	1464.3	1595.1	12
Avg. uses per year	77.1	120.5	8
Uses past 30 days	7.5	10.5	8
Cannabis			
Age of First Use	16.8	3.99	12
Lifetime days of use	2579.1	3297.03	12
Avg. uses per year	122.25	153.8	8
Uses past 30 days	15.8	12.8	8
Cocaine/crack			
Age of First Use	23.7	6.5	12
Lifetime days of use	2100.3	1548.5	12
Avg. uses per year	186.33	109.6	12
Uses past 30 days	14.3	8.4	12
Amphetamines			
Age of First Use	28	13.2	4
Lifetime days of use	15.25	17.03	4
Avg. uses per year	150	0	1
Uses past 30 days	1	0	1
MDMA			
Age of First Use	23.5	6.02	6
Lifetime days of use	16.16	18. 7	6
Avg. uses per year	1	0	1
Uses past 30 days	1	0	1
Tobacco			
Age of First Use	17.4	6.5	11
Lifetime days of use	52657.2	61308	11
Uses past 30 days	365	0	9
Cigarettes/Day	11.7	6.64	9

Supplement Table 2: Principal component analysis (PCA) on the double delta VAS scores. Loadings smaller than 0.6 are not depicted.

Factors	1	2	3	4	5	6
Happy		0.911				
Rush				0.869		
High						
Euphoria			0.855			
Excited		0.802				
Anxious				0.671		
Energetic			0.803			
Mind-racing			0.654			
Alert	0.754					
Bored		-0.628				
Interested						0.929
Urge for cocaine	0.852					
Desire cocaine	0.851					
Crave cocaine	0.940					
Want cigarette				0.655		
Want alcohol					0.919	
Want other drug			0.695			

Supplement Table 3a.: [^{18}F]Fallypride BP_{ND} on neutral and cue day in high craving group in Regions of Interest (ROI) (N=6/group).

Region	Mean ± SD		P values
	Neutral Cues	Cocaine Cues	
limbic striatum	32.63± 4.20	29.12± 3.81	0.019
Associative striatum	35.87± 5.14	32.72± 6.16	0.008
Sensorimotor striatum	41.46± 5.89	36.06 ± 1.55	0.004
Amygdala	2.72 ± 1.01	2.34 ± 0.82	0.040
Hippocampus	1.06 ± 0.47	0.96 ± 0.40	0.133
Right Hippocampus	0.98 ± 0.29	0.86 ± 0.253	0.025

Values represent Mean ± SD.

Supplement Table 3b. :[^{18}F]Fallypride BP_{ND} on neutral and cue day in low craving group in Regions of Interest (ROI) (N=6).

Region	Mean ± SD		P values
	Neutral Cues	Cocaine Cues	
limbic striatum	31.22± 5.86	31.31± 5.54	0.96
Associative striatum	3416± 3.29	34.63± 3.21	0.59
Sensorimotor striatum	39.30± 4.41	41.84 ± 5.92	0.10
Amygdala	2.83 ± 0.60	2.81 ± 0.57	0.83
Hippocampus	1.06 ± 0.25	0.96 ± 0.27	0.54
Right Hippocampus	0.85± 0.26	0.87± 0.34	0.63

Values represent Mean ± SD.

Supplement Table 4: The relationship between VAS factors and percent changes in [^{18}F]fallypride BP_{ND} in ROIs (N=12).

	Limbic	Associative	Sensorimotor	Hippocampus	Amygdala
Crave Factor					
Pearson Correlation	0.581	0.589	0.675	0.213	0.258
P value	0.048	0.044	0.016	0.506	0.417
Factor2					
Pearson Correlation	0.238	0.359	0.216	0.332	0.392
P value	0.456	0.252	0.500	0.291	0.208
Factor3					
Pearson Correlation	-0.144	-0.129	-0.004	0.120	0.363
P value	0.656	0.689	0.989	0.710	0.246
Factor4					
Pearson Correlation	-0.436	-0.510	-0.312	0.505	0.148
P value	0.157	0.090	0.323	0.094	.646
Factor5					
Pearson Correlation	0.079	0.255	0.301	-0.231	-0.357
P value	0.806	0.425	0.341	0.470	0.255
Factor6					
Pearson Correlation	0.033	-0.168	-0.304	-0.204	-0.340
P value	0.919	0.601	0.336	0.525	0.279

General Discussion

This study provides the first evidence of cue-induced DA release in the amygdala and hippocampus in humans, and replicates the previously reported effect in the striatum. This response was only observed in the higher craving subgroup of our participants. A DA response was observed in all the striatal functional subregions (limbic, associative and sensorimotor) and was directly associated with the level of craving in these regions.

Cue-induced DA response in amygdala and hippocampus

Both amygdala and hippocampus have been thought to play an important role in the acquisition and expression of learned associations between reward and its predictive stimuli, influence the ability of motivationally salient stimuli to elicit and sustain interest and facilitate the selection of situation appropriate behavioral responses (Robbins and Everitt, 2002, Phillips et al., 2003, Goto and Grace, 2008, Robbins et al., 2008, Shohamy and Adcock, 2010). Animal studies have demonstrated that lesioning or inactivation of the amygdala (Meil and See, 1997, Kantak et al., 2002) or hippocampus (by TTX)(Rogers and See, 2007) diminishes behavioral responses to drug cues, whereas electrical stimulation of these regions increases drug-seeking behavior (Vorel et al., 2001, Hayes et al., 2003). Studies in humans, using brain glucose uptake (Grant et al., 1996) cerebral blood flow (Childress et al., 1999) and fMRI BOLD signals (Wexler et al., 2001) have demonstrated that both the amygdala and hippocampus are highly active when drug users are exposed to drug related cues. Taken together, these observations highlight the need to explore the neural substrates that relates to the activity of these regions and ultimately affects drug-seeking behavior.

The effect we found in the amygdala is consistent with findings from animal studies that have demonstrated a cue-induced DA response in the amygdala (Weiss et al., 2000). Altering DA transmission in the amygdala through the use of DA agonists and antagonists has been shown to influence reward-approach behavior in animals (Hitchcott et al., 1997) (Berglind et al., 2006). Accordingly, the observation of cue-induced DA signal in amygdala in humans could potentially indicate a similar effect in influencing drug-seeking behavior. Our finding of a dopaminergic response to cocaine cues in the

hippocampus, though, is a unique finding in both animal and human literature. Although human neuroimaging studies provide evidence of cue-induced activation in the hippocampus (Grant et al., 1996, Kilts et al., 2001, Wexler et al., 2001), to date, the neural substrates that are related to this activation remain relatively unknown. This noted, emerging evidence supports the role of hippocampal DA transmission in the formation and reactivation of reward related memories (Shohamy and Adcock, 2010).

In the current study, we did not differentiate between sub-regions within the amygdala and hippocampus. It is important to note, though, that these regions consist of heterogeneous nuclei that might have independent functions and different contributions to learning the aspects of cue-reward association. Balleine et al have suggested that the two main nuclei in amygdala, the Basolateral (BLA) and Central Nucleus (CeN), do not work serially but rather independently, on aspects of reward processing. The BLA has been implicated in goal directed behavior while the CeN has been implicated in habit learning (Lingawi and Balleine, 2012). While the PET resolution precluded the analysis of the specific response of these separate nuclei, it is worth noting that they often work in parallel and simultaneously (LeDoux, 2007). Thus, the amygdala as a whole remains a general functional unit in the processing of cue-reward association. Similarly, there is a small but steadily increasing body of literature ascribing independent roles to the hippocampus nuclei in drug-seeking behavior. Stimulation of the ventral subiculum can prompt drug seeking in rats (Vorel et al., 2001, Lasseter et al., 2010), whereas the dorsal subiculum plays a significant role in the reinstatement of cocaine seeking by cocaine itself (Martin-Fardon et al., 2008). Furthermore, some authors have suggested that independent memory circuits exist between hippocampus nuclei and striatal regions. The ventral subiculum forms a circuit with the VS shell that is implicated in retrieval of cue-contingencies, whereas the dorsal subiculum is connected with the VS core. The function of ventral subiculum-VS core is mostly unknown but it is proposed to influence control of spatial behavior (Pennartz et al., 2011) and the influence of contextual cues (Selden et al., 1991).

We found a stronger effect in the right hemisphere both in the amygdala and hippocampus. Some studies have provided evidence that memory formation and

activation in medial temporal lobe is coded differently in left and right hemispheres (Martin, 1999). These studies suggest that the right hemisphere is mainly activated in response to visual stimuli, whereas the left hemisphere is mostly implicated in verbal memory. Also, the right hemisphere has been shown to be more active during tasks that involve attention and arousal (Martin, 1999). Studies that have specifically investigated lateralization of amygdala and hippocampus also indicate that for both regions, the right hemisphere is relatively more active in response to visual memory (Markowitsch, 1998, Costafreda et al., 2008) and that the right hippocampus demonstrates dominant activity when both visual and verbal memory are involved (Squire et al., 1992). Our findings are in accord with the above proposition and could be explained by the prolonged exposure to visual cues. However, the laterality effect is not consistent across studies (Baas et al., 2004) and need to be further investigated in future.

In our study, individual differences in craving did not predict the magnitude of DA response in the amygdala and hippocampus. This observation differs from what we found in the striatum and is not in line with some of the previous imaging studies measuring transmitter non-specific activations (Grant et al., 1996). One interpretation is that craving is more closely related to DA release in the striatum than in the amygdala and hippocampus. Alternatively, there is less noise in the [^{18}F]fallypride signal in the striatum than other regions. Our study, therefore, may have had sufficient resolution to detect a large group difference but not the finer nuances of individual differences.

Striatum-Amygdala-Hippocampus as a single functional unit

The amygdala, hippocampus and striatum are anatomically distinct structures and their explicit role in processing aspects of cue-reward learning and-behavior has been a subject of study for decades (Robbins et al., 2008). However, recent findings have provided a novel view indicating that these regions should rather be considered as a single functional unit (Cacciapaglia et al., 2012). This proposition suggests that the complex cue-induced motivational and subjective states that lead to drug-seeking behavior can only be the result of interactions between the structures of a common limbic-striatal system. Several lines of evidence support this proposition: Firstly, limbic and striatal regions are highly interconnected. There are extensive afferent fibers from the

amygdala and hippocampus to the NAcc and stimulation of these pathways leads to increased DA response in NAcc (Floresco et al., 2001). Specific function for some of these pathways has been reported. For example, Ventral subiculum-VS shell has been implicated in retrieval of cue contingencies (Pennartz et al., 2011). The amygdala and hippocampus have also been shown to be highly interconnected and the amygdala has been suggested as an important structure to modulate the memory related processes in the hippocampus (Tsoory et al., 2007) (Huff and Rudy, 2004). Secondly, animal studies that have investigated the functional importance of these connections have reported that lesions to the connecting pathways between these structures can also disrupt drug-seeking behavior (Burns et al., 1993). Finally, considerable commonality between the functions of striatal and limbic regions exists and each of these regions contributes to aspects of cue-reward learning and response. For example, lesions of NAcc Shell, CeN in amygdala and ventral subiculum all disrupt response to drug cues in animals (Rogers and See, 2007, Lingawi and Balleine, 2012). Our finding that drug cues can elicit DA response in all these structures further strengthens the proposition that the striatum, amygdala and hippocampus might function as components of an integrated system and that DA is one of the key signals that regulates this collaborative response. However, the precise mechanism through which these structures interact and to what extent each structure regulates the other remains unclear. Future studies are needed to address this issue.

Group difference: DA response in high vs. low cravers

Cue-induced DA increase occurred only in the high craving subgroup of our participants. There could be several reasons for this finding. First, the videos might resemble the drug experience of only some participants. Previous studies indicate that personalized cues are more potent in inducing drug craving in drug users (O'Brien et al., 1979, Staiger and White, 1991, Conklin et al., 2010). These observations encouraged the more recent studies to use measures such as autobiographical scripts for cue-induced craving. Considering this, the videos used in our study were designed for a Canadian viewer (e.g. using hockey scenes). We also used the autobiographical script to maximize the extent to which participants would relate to the videos. Although we believe our videos, together with the autobiographical script and the presentation of paraphernalia,

provided a rich cue microenvironment, some participants might still be substantially non-responsive to non-personalized cues and environments. It could be speculated that over time, practices and rituals might become more personalized. The marginally negative association between craving and years of use is at least consistent with this proposition.

Second, the perceived opportunity to access drugs after the exposure to drug cues and/or active intention to inhibit the craving could be two factors that might have influenced our group difference. A recent body of research demonstrates that the expectancy to receive actual drug-related reward after cue exposure can modulate (i) subjective appetitive states for drugs (Wertz and Sayette, 2001), (ii) drug-seeking behaviors (Hogarth et al., 2007), and (iii) brain activations (McBride et al., 2006) (Kringelbach and Rolls, 2004, Wilson et al., 2005, Wilson et al., 2008). Furthermore, a conscious decision to avoid drug consumption has also been reported as an important factor that can affect cue-induced appetitive states for drugs as well as regional brain activity (Prisciandaro et al., 2012) . This effect has been demonstrated both when the inhibition was driven by an internal incentive (treatment seeking individuals) and when driven by external circumstances (unavailability of the drug). Interestingly motivated and unmotivated cigarette smoker quitters showed different brain activity patterns in response to cues (Wilson et al., 2012). Moreover, when non-treatment seeking cocaine dependent users were asked to actively inhibit their craving when exposed to cocaine related cues, a similar pattern was observed (Volkow et al., 2010). Therefore, our observed group differences may have resulted from anticipation and expectancy of using cocaine on the evening after the cocaine cue day scan as well as from the intention to inhibit the subjective craving in a research environment given the demands of the study. Thus we can argue that the DA response could have been suppressed or augmented according to the two mentioned factors. Nevertheless, the objective of our study was to test whether cues – by themselves – are able to elicit a DA response; whether this effect could be modified by other factors could be the subject of future studies. Furthermore, previous studies report that delayed opportunity of drug access after presentation of cues dissociates expectancy effect from DA response (Roesch et al., 2007). In our study, the participants were aware that they would be asked to not leave the research unit until two hours after the scan is over. This might have lowered the expectancy of a prospective

reward (drug). Finally the effect of expectancy in altering regional activity has been mostly implicated in frontal regions, i.e. "control networks", including OFC, prefrontal, cingulate, inferior parietal lobe and thalamus (Volkow et al., 2011). Whether expectancy to receive reward influences limbic regions (e.g., hippocampus and amygdala) or DA plays a role in this procedure remains unknown.

A third possibility is that individual differences in cue responsivity could represent a trait. Recent animal research has emphasized that there are marked individual differences in animals' approach to reward. Robinson et al. propose that individual differences are an important factor to determine approach to reward or reward associated cues. They reported that conditioned stimulus becomes attractive and elicits approach behavior in some rats whereas for others the reward related cue is only a predictive signal and does not gain incentive salience (Robinson and Flagel, 2009). They called these groups of rats sign-trackers and goal-trackers respectively. Moreover, cue-induced accumbens DA release was only seen in the sign trackers (Flagel et al., 2010). Flagel et al also report that administration of DA antagonist disrupted acquisition of conditioned response in sign tracking but not the goal tracking rats. This observation suggests that DA might mediate distinct neural systems in CR learning of sign and goal trackers. Since all our participants showed an increased subjective craving to the cocaine cues but the DA response was only observed in the higher craving subgroup, it could be speculated that these higher craving individuals attribute incentive salience to the presented cocaine cues which is associated with higher DA signalling. This finding might be the first evidence of sign tracking behavior in cocaine users and might characterize the individuals who are more susceptible to environmental cues.

Regional differences in BP_{ND} binding

Although a DA response was observed in all the defined striatal regions, this response showed regional variations. The robust effect found in the posterior putamen overlaps with the dorsal striatum region identified in PET [^{11}C]raclopride studies (Volkow et al., 2006, Roesch et al., 2007). In studies conducted in laboratory rats, cue-induced DA release in dorsal striatum occurs after relatively extensive cocaine use histories (Ito et al., 2002). In comparison, Boileau et al. report DA response to drug-

paired cues in healthy volunteers after administration of only three doses of d-amphetamine. (Boileau I and et al., 2006). The more widespread effect in our study could reflect (i) the participants' intention of use that evening and the ability of the cues to elicit a combination of processes that regulate flexible goal-directed seeking behavior and inflexible stimulus-response habits, (ii) the use of a different tracer plus higher resolution camera, or (iii) methodological differences with previous human studies such as the presentation of paraphernalia and autobiographical scripts in our experiment.

Limitations

Our findings should be interpreted in the light of the following considerations. Firstly, our sample size was modest and the results should be generalized with caution. Secondly, in this study, 10 out of 12 participants were male, which prevented us from making gender comparisons. Addressing gender effects in future studies is necessary. Third, our scans were always administered in a fixed order (neutral day first, followed by cocaine day) to avoid the carry over effect of cocaine and to avoid drug-paired environment effects (i.e., the scan unit) for the neutral day. However, the fixed order might raise the concern that our design has not benefited from counter balancing and might have affected both subjective and dopaminergic responses on the cocaine cue day. We tried to minimize the effect of novelty elicited by the PET environment by familiarizing the participants with the procedure, and periodically measuring the physiological indexes of stress such as heart rate and blood pressure on both days. Furthermore, previous findings support test retest reliability for fallypride receptor binding in repeated scans (Mukherjee et al., 2002). In addition, the heightened DA response was only observed in a subgroup of our participants (high-cravers), which indicates that the observed effect is independent from the scan order. Hence the non-counter balanced design could have undermined the effect we found in our study, which further supports our findings. Fourth, given the long duration of the scan (150 minutes) some videos were repeated 2-3 times and this might have caused boredom. Even so, we found the higher craving and higher DA responses on the cocaine cue day and we might have found a more robust response had the videos not been repeated.

Conclusion

To our knowledge this study provides the first evidence of drug cue-induced DA release in the amygdala and hippocampus in humans. The preferential induction of DA release in cue-responders suggests that these aspects of the limbic reward network might contribute to drug-seeking behavior and could be considered as a target for modulation for prevention of relapse. Furthermore individual differences observed in drug-cue responsivity could indicate regulating factors such as habituation to personalized cues, perceived opportunity to access drugs or active inhibition of craving or a personality trait. Future studies are necessary to investigate the regulatory effect of these factors on behavioral and neurochemical response to cues.

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MONTREAL NEUROLOGICAL INSTITUTE & HOSPITAL
MCGILL UNIVERSITY

Departments of Psychiatry and Neurology & Neurosurgery

Title of the Project: Cue-related changes in striatal and extra-striatal dopamine release.

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1. REASON FOR THE STUDY

Cocaine is an extremely addictive drug. Desire for cocaine can come to dominate a person's life, and both the personal and medical consequences can be overwhelming. Since a common trigger of this desire is exposure to drug related cues we plan to study this effect further.

In the present study, we will measure the effect of drug related cues on the brain chemical dopamine. Previous studies suggest that dopamine has an important role in regulating responses to natural rewards and the cues that predict them. To investigate whether a similar association is present for a drug like cocaine, participants will watch cocaine themed videos while self-reported mood, heart rate and other physiological responses are measured. Dopamine release will be measured with a brain imaging method, and we are interested in how the observed changes in the brain are related to how people feel.

2. PROCEDURE

Your participation in this study will involve 4 sessions on separate days. The first session involves a clinical interview and a medical examination. You will then have two Positron Emission Tomography (PET) sessions, and finally a Magnetic Resonance Imaging (MRI) session.

A) Initial Assessment

The first session will involve an interview with one of the investigators. You will be asked to complete some paper and pencil questionnaires, and then the investigator will perform an interview of approximately three hours duration. The purpose of this interview is to gather background information about you and your family, and you will be asked about personal and family histories of depression, alcohol or drug problems, and other psychological disorders.

Following the assessment, those who participate in the study will come to the research ward for two separate PET test sessions and one MRI session. Before each PET test day starts, a urine drug screen will be conducted for drugs of abuse (cannabis {marijuana, hash}, amphetamines {e.g., speed, meth, uppers}, cocaine, benzodiazepines {e.g., valium, ativan}, barbiturates {chloral hydrate}, opiates {e.g., morphine, codeine, heroin}). This is done to avoid possible interactions between the experimental manipulation and substances that the volunteer might have used. For participants who are women, a urine pregnancy test will be conducted at the same time. Study days will only proceed if the pregnancy test is negative.

PET Test day 1: you will undergo a PET scan and will fill out various mood scales while viewing a neutral video. A series of small blood samples will be drawn (2 x 2 tbsp), and cardiovascular activity will be monitored throughout.

PET Test day 2: you will undergo a PET scan and will fill out various mood scales while viewing a cocaine themed video. During this process you will rate the subjective effects of the videos on various scales. A series of small blood samples will be drawn (2 x 2 tbsp), and cardiovascular activity will be monitored throughout.

B) Positron Emission Tomography

The PET scans will be done between 11:00 and 17.00, the scan will last about three and a half hours (approximately 12:30 – 16:00). During this time, you will be asked to lie on a couch, watch videos, and perform simple tasks. A fine needle-catheter will be inserted into an arm vein for the administration of small amounts of the radioactive tracer, fallypride, and to draw venous blood samples. After two hours in the scanner you will be given a half hour break, then return for the final hour of scanning.

1) Avoid excessive fluid intake on the day of each PET scan, as you will be immobile for up to two hours during the PET scan.
2) The tracer that you will be administered is fallypride, which is labeled with the short-lived radioactive atom, fluorine 18 [18-F] (physical half-life = 110 minutes). The total dose administered to you will be 5 millisieverts (mSv).
3) During the PET study, a number of venous blood samples will be drawn from the catheter to measure levels of the tracer. This will be equivalent to about 60mls of blood, equivalent to 10 teaspoons per PET scan session.
4) All procedures during the PET study will be carried out by a qualified nuclear medicine technician, and supervised by a qualified nuclear medicine physician. You will be able to communicate with the technician at all times.

C) Magnetic Resonance Imaging

You will be asked to lie on a couch that will be moved into a cylindrical opening where pictures of your head will be taken during a period of 30 minutes. The MRI machine will be quite noisy during the scan. To reduce the noise, you will be given earplugs. You will be able to communicate with the technician during the procedure. Because skin patches for transcutaneous medication administration can cause local overheating during the MRI study, you will be asked to remove any such patch before the procedure. You should bring a new patch with you if you need to re-start the medication immediately after the study

D) Follow-Up

Following completion of the brain imaging, participants will be invited to take part in a series of interviews conducted one day, one week, one month, three months, six months, and 12 months after the study. During these interviews, participants will complete questionnaires about their mood and any difficulties they may have experienced related to depression, alcohol or drug problems, or other psychological problems.

3. TIME COMMITMENT

The study involves a time commitment of approximately 20 hours (excluding follow-up interviews). This includes: (a) the interview plus medical exam (up to 5 hours), (b) PET test session 1 and 2 (up to 7 hours each, from 11am-6pm) and (e) an MRI session to obtain a structural image of your brain (1 hour).

The follow-up interviews will take approximately 1 hour per interview, up to a total of 7 interviews (24 hours, one week, one month, three months, six months and one year following the last PET session).

4. CONTRAINDICATIONS

A) For PET Study

The following are contraindications for this procedure.

1) Pregnancy or Breast Feeding
2) Under 18 years old
3) Previous radiation absorbed doses received within the past (12 months) that would lead, with inclusion of this study, to an aggregate radiation absorbed dose exceeding 5 mSv.

B) For MRI Study

The following are contraindications for this procedure.

1) Cardiac Pacemaker
2) Aneurysm Clip
3) Heart/Vascular Clip
4) Prosthetic Valve
5) Metal Prosthesis
6) Pregnancy
7) Claustrophobia
8) Transdermal Patches (Must be removed prior to scanning. Subject is advised to bring an additional patch to reapply post-scanning)

5. ADVANTAGES OF THE PROPOSED STUDY

There is no advantage to the participants in being involved in this study except for the remote possibility that the initial screening may reveal a treatable condition. In the long run, it is hoped that this study may reveal more information about addiction.

Both PET and MRI studies are tests, not treatments. It is hoped that the information obtained will help our understanding of the function of the human brain. This may, in the long term, help the diagnosis and treatment of neurological and other brain disorders.

Information about the Montreal General Hospital Drug Dependency Treatment Unit is available for cocaine users who would like to receive treatment for their cocaine use. Potential patients are asked to contact the Addictions Unit secretary, Patty Vlahos, at 934-1934 x42399 any time from 8am - 4pm. The costs of treatment are covered by Medicare.

6. DISADVANTAGES OF THE PROPOSED STUDY

A) For PET study

1) Some discomfort may be caused by insertion of the fine needle-catheter into the vein, as well as immobility on the couch.
2) The main **RISK** of participating in this study is exposure to radiation from the short-lived tracer substance injected into your body. The administered radioactive material will expose your body to a maximal dose of 5 mSv, according to our best scientific estimates. This level of radiation dose is about two times that you receive annually from natural background radiation (0.9 - 2.2. mSv) in various regions of North America. It is also 25% of the current average annual dose limits allowed for those who work in a high radiation environment, such as nuclear medicine technicians. The degree of **RISK** associated with exposure to an additional 5 mSv of radiation is thought to be very low. This amount of additional radiation may increase the risk of fatal cancer by about 2 in 10,000 during a lifetime, while the current overall risk of fatal cancer is about 2,300 in 10,000. Similar risks, equivalent to those from the dose you are receiving, are associated with:
   (a) smoking 2 packs of cigarettes during a lifetime (cancer, heart disease)
   (b) driving 2,000 miles by car (accident)
   (c) flying 20,000 to 60,000 miles by air (accident)
   (d) living 100 days in New York or Boston (air pollution)

Please note that the substance being injected to perform the PET study is not currently approved for general human use in Canada. However, its use for research purpose has been reviewed and allowed by Health Canada.
Additional information available upon request

B) For MRI study
During this study, you will be exposed to a strong magnetic field. No long-term negative side effects have been observed from this type of study. As mentioned above, the MR is very noisy and you will be given earplugs to reduce this effect.

7. EFFECTS OF PARTICIPATION IN THIS STUDY ON YOUR TREATMENT

Positron emission tomography, magnetic resonance imaging does not interfere with any treatment or other diagnostic tests.

8. CONFIDENTIAL NATURE OF THIS STUDY

A hospital chart will be opened in your name. It will only contain information related to your standard blood tests. It will not contain information about your substance use. Information about your personal history, including substance use, will be kept in a separate file accessible to the Research Team only. This file will be listed by a code

number and be kept in a locked cabinet. The information will be kept confidential, unless otherwise specified by law. No personal information will be released to other parties without your written approval. Your name, date of birth, address and telephone number may have to be forwarded for review by Health Canada. The subject should be aware that the Research Ethics Board or Quality Assurance Officers duly authorized by it may access study data. In addition, Canadian Nuclear Safety Commission could also be granted access to research files.

9. INCIDENTAL FINDINGS

MRI and PET research scans are not subject to clinical review. However, any incidental findings noted by the researcher will be communicated to you and, upon your request, to your physician.

10. DISCONTINUATION OF THE STUDY BY THE INVESTIGATOR

At any time during the testing, the investigators have the right to terminate the study for any reason.

11. COMPENSATION

Participants who complete the study will receive a cheque for $300 in compensation for their time and inconvenience. If, during the initial interview, the study investigators decide that a subject does not meet the entry criteria, they will receive $25. If a subject decides to withdraw from the study, they will receive partial compensation based on time spent in the study.

Participants who take part in the follow-up interviews will receive $20 for time and inconvenience associated with each interview.

12. SUBJECTS' RIGHTS

If you have any comments or concerns, or need assistance regarding your participation as a research subject in this project, please contact the Principle Investigator, Dr. Leyton, tel. 514-398-5804 or contact the MNH Patient's Committee, room 354, tel. 514 398 5358. If you have any questions regarding your rights as a research subject and you wish to discuss them with someone not conducting the study, you may contact the Montreal Neurological Hospital, Patient Ombudsman at 514 934 1934, ext 48306. You will be informed of any new information that might appear during the course of the study that could affect your willingness to participate.

13. WITHDRAWAL FROM THE STUDY

Participation in this research project is voluntary and subjects may withdraw at any time, including during the procedure without prejudice. Data accumulated up to the time of your withdrawal will be kept in use for research purposes

1.1.1.1 DECLARATION OF CONSENT

Title of Project**: Cue-related changes in striatal and extra-striatal dopamine release.**

Place of Testing: Montreal Neurological Institute / McGill University Health Centre

I, _____, have read the above description with

one of the above investigators, _____.

I fully understand the procedures, advantages and disadvantages of the study which have been explained to me. I freely and voluntarily consent to participate in this study.

I hereby certify that I have not participated in a PET investigation anywhere before (within the past Twelve (12) months).

Further, I understand that I may seek information about each test either before or after it is given, that I am free to withdraw from the testing at any time if I desire, and that my personal information will be kept confidential.

SIGNATURE _____ _____ _____

SUBJECT ***DATE*** ***CONTACT NO.***

SIGNATURE _____ _____ _____

INVESTIGATOR ***DATE*** ***CONTACT NO.***

Magnetic Resonance Imaging Questionnaire

Subject last name:_____ First name: _____

Date of birth:_____
dd / mm / yy

Sex: F ☐ / M ☐

Previous surgery?	NO	YES	If yes indicate the type
Head			
Heart			
Eyes			
Abdomen			
Extremities			
Spine			

Others:_____

Do you have a:	NO	YES	
Cardiac Pacemaker / Defibrillator			
Cochlear implant or implanted hearing aid			
Implanted insulin pump			***If yes, must be removed for scan***
Coloured contact lenses			***If yes, must be removed for scan***
Transdermal delivery system (e.g. patch)			***If yes, must be removed for scan***
Body piercing			***If yes, must be removed for scan***
IUD			
Foreign metallic objects (e.g. bullets or metal splinters)			
Permanent make-up / tattoos			
Ocular implants or devices			▶
Cardiac valve prosthesis			▶
Neurostimulator			▶
Artificial limb or joint			▶
Implanted orthopedic device			▶
Penile implant			▶
Aneurysm Clip			▶
Filter, catheter or stent in a blood vessel			▶
Shunt (programmable)			▶

Specify type

	NO	YES
Are you pregnant?		
Have you ever been injured by a metallic piece? (e.g. in your eyes)		
Have you ever undergone Magnetic Resonance Imaging?		
If yes when: _____		
Do you suffer from claustrophobia?		

_____ _____
Subject signature Date (dd-mm-yy)

_____ _____
Physician / Researcher signature Date (dd-mm-yy)