Neuropsychopharmacology (2013) 38, 1780–1788

www.neuropsychopharmacology.org

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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Drug-related cues are potent triggers for relapse in people with cocaine dependence. Dopamine (DA) release within a limbic network of striatum, amygdala and hippocampus has been implicated in animal studies, but in humans it has only been possible to measure effects in the striatum. The objective here was to measure drug cue-induced DA release in the amygdala and hippocampus using high-resolution PET with [^{18}F]fallypride. Twelve cocaine-dependent volunteers (mean age: 39.6 ± 8.0 years; years of cocaine use: 15.9 ± 7.4) underwent two [^{18}F]fallypride high-resolution research tomography–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. 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: $r = 0.675$, $p = 0.016$). 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 among high-craving responders suggests that these aspects of the limbic reward network might contribute to drug-seeking behavior.
Neuropsychopharmacology (2013) **38,** 1780–1788; doi:10.1038/npp.2013.77; published online 1 May 2013

Keywords: addiction; craving; reward; striatum; limbic; conditioning

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 amygdala 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*,

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Received 21 November 2012; revised 23 March 2013; accepted 27 March 2013; accepted article preview online 1 April 2013

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

MATERIALS AND METHODS

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 (Supplementary Table 1). No participants were currently seeking treatment for their substance use problems or planning to quit within the month following the study. 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 h 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 given a urine pregnancy test before each PET session; none tested positive (Assure FastRead hCG Cassette, Conception Technologies, San Diego, California, USA). As gonadal hormone levels fluctuate during the menstrual cycle and these changes are thought to influence reward-related neurotransmission (Becker, 2009), female participants were tested during their follicular phase when estradiol and progesterone levels are lower and more stable. Menstrual phase was verified by self-report and all were tested in the first 7 days of their cycle.

On the neutral cue session (Figure 1), participants developed, 2 h 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 for 30 min. 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 min. Subjects were then shown a 10-min 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 subjectively 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 rock-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 for 15 min. For the following 15 min, 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 and one T_1-weighted

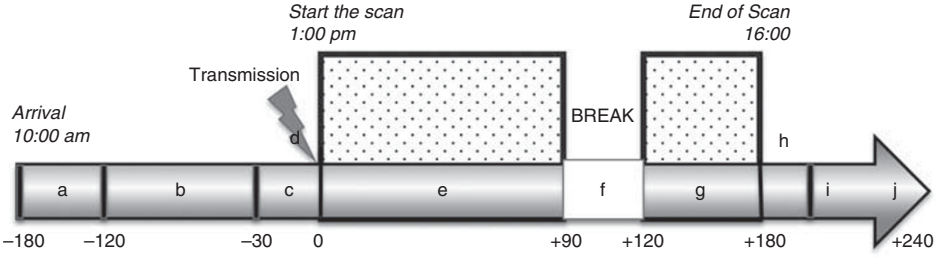

Figure 1 Test day procedures and timing. Time points are defined according to start of emission scan (time point 0). a: Arrival at the PET unit, baseline measurements, and urine drug test. b: Develop autobiographical script, manipulate paraphernalia, watch video highlights (context different on neutral and cue day as described in Supplementary 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.

MRI session for PET/MR co-registration. PET sessions consisted of a bolus injection of 3.30 ± 0.24 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; please see Supplementary Methods) were calculated (Cunningham *et al*, 1991; Innis *et al*, 2007) using the Simplified Reference Tissue Model (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 (http://www.turkupetcentre.net/). Regional BP_{ND} values were weighted with the volume size when combining both hemispheres (see Supplementary Section for additional details).

ROI Analysis

We focused on a restricted number of *a priori* defined ROIs 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) (http://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 min before the start of the scan and then every 30 min 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 General Linear Model 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 extrastriatal regions avoided the homogeneity issue but increased the risk of type I errors due to failure to correct for multiple testing. As 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 $p \leqslant 0.05$. 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 (Supplementary Table 1) but reported cocaine as their drug of choice. No participants were currently seeking treatment for their substance use problems.

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, as 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 (Supplementary 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.

Table 1 Characteristics of Research Participants ($N=12$)

Characteristics	Value (mean ± SD)
Age (years)	39.5 ± 8.0 (range: 31–48)
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 (range: 3–25)
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.

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

The four-way group × session × ROI × hemisphere ANOVA of BP_{ND} values yielded a three-way group × session × 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]fallypride BP_{ND} among the high-craving subjects (Figure 3; Supplementary Figure 1; Supplementary Table 4). Among subjects exhibiting high-craving factor scores, exposure to the cocaine cues, compared with 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 (Least Significant Difference *post hoc*: $p=0.047$; *t*-test: $t_{(5)}=3.15$, $p=0.025$). These effects were not seen in subjects with low-craving scores ($p\geqslant 0.1$) (Figure 3; Supplementary Table 4).

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

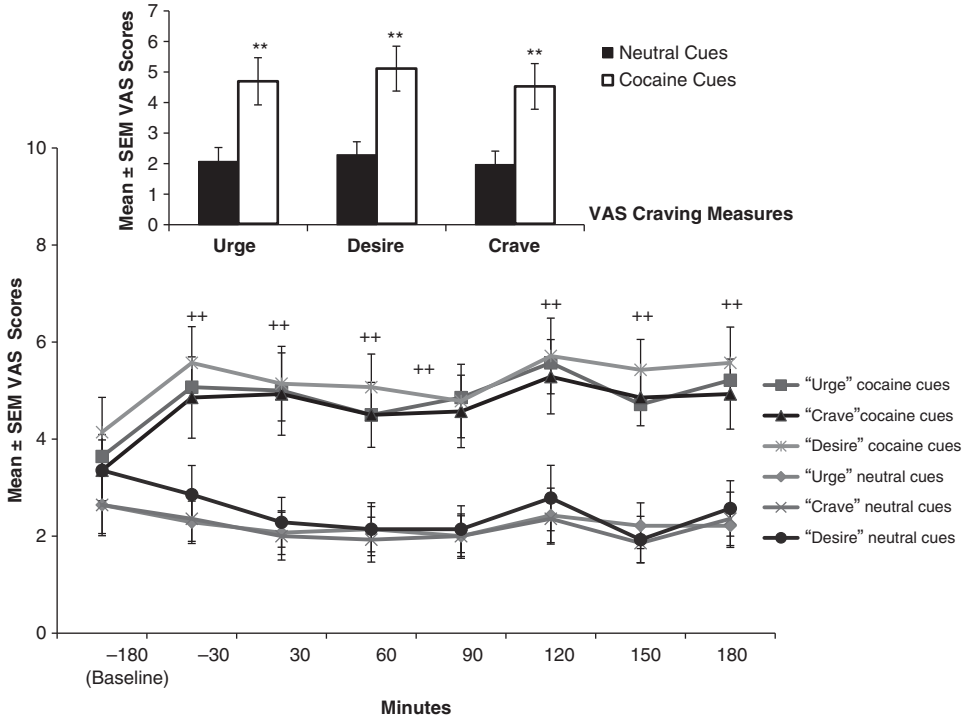

Figure 2 Changes of mean craving scores of self-report visual analog scales on neutral and cocaine cue day. Time points are defined according to start of emission scan (time point 0). Cue exposure starts approximately 1 h after baseline time point. Error bars represent standard errors of the mean (All *t*-values > 3.5, df = 11, ***p* values < 0.005).

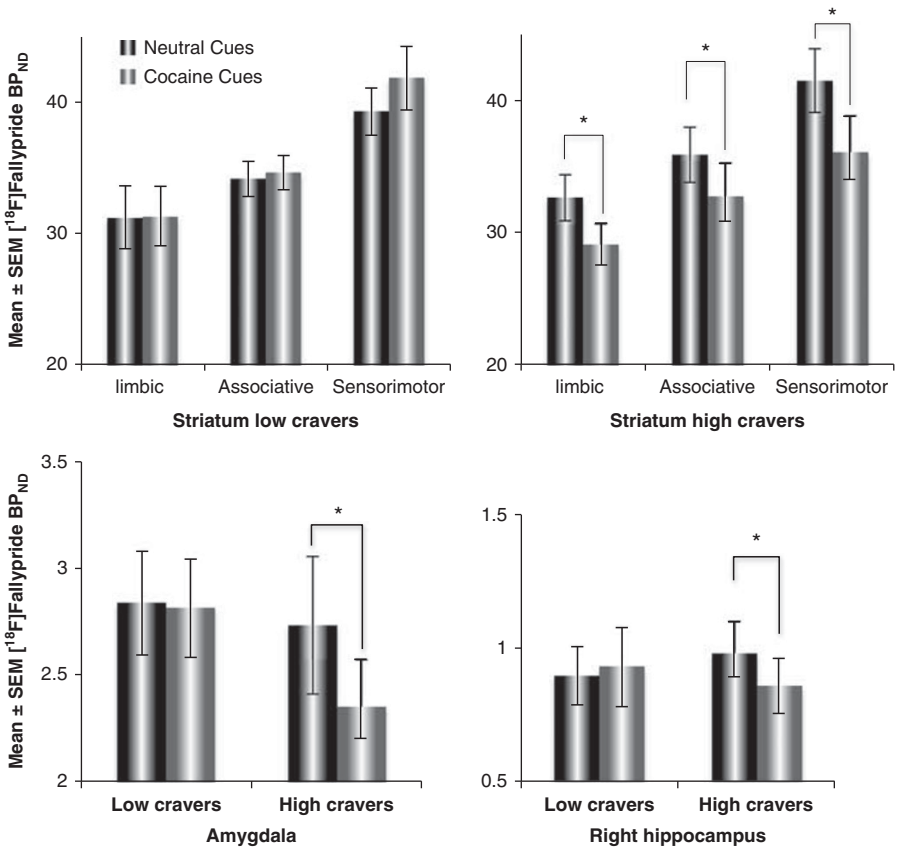

Figure 3 [18F]Fallypride BPND on neutral and cocaine cue days in subjects with high- and low-craving factor scores Top: striatal regions. Bottom left: amygdala. Bottom right: hippocampus. Values represent mean ± SEM. *$p < 0.05$.

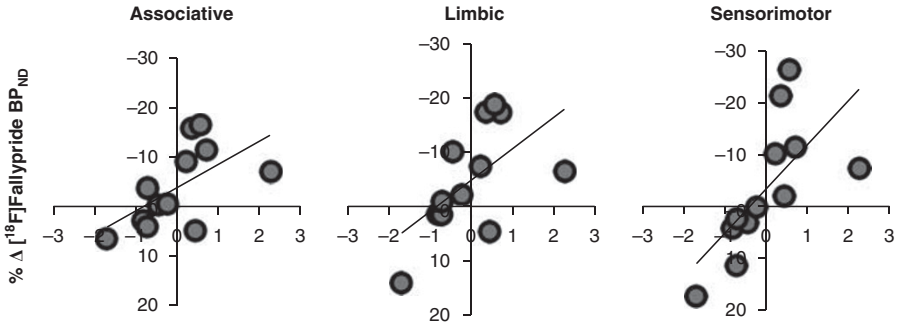

Figure 4 The relationship between changes in cue-induced craving factor score (*x* axis) and percent changes in [^{18}F]fallypride BP_{ND} (*y* axis) across the test sessions in striatal ROIs ($N = 12$). Associative striatum ($r = 0.589$, $p = 0.044$), ventral limbic striatum ($r = 0.581$, $p = 0.048$),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

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 have 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 and Phillips, 1997). Our own study raises the possibility that cue-induced amygdalar DA release has 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 and White, 1993; Eichenbaum, 2013; Dickerson and Eichenbaum, 2009). Several studies have demonstrated that DA transmission facilitates hippocampal synaptic long-term potentiation (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, as 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 (eg, 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 with the striatum, DA 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 and Robbins 2005). Third, our PET scans were 3 h in duration (time post-tracer injection). There is broad consensus that 60–90 min is sufficient to detect effects outside of the basal ganglia (ie, 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 2 h (Ceccarini *et al*, 2012); empirical data indicate that tracer equilibrium is clearly achieved by 3 h (Vernaleken *et al*, 2011). The present study plus work conducted elsewhere further confirm that scans of 180–210 min 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; eg, 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 before the neutral test session. This benefit of the design was considered reasonable as [^{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.

ACKNOWLEDGEMENTS

We thank R Fukasawa, G Sauchuck, and S Mattei for technical assistance at the PET unit; D Jolly and M Kovacevic for preparation of radiotracers. Funding for the study came from an operating grant to ML from the Canadian Institutes of Health Research (CIHR, MOP-36429).

DISCLOSURE

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)