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Dysfunctional Amygdala Activation and Connectivity With the Prefrontal Cortex in Current Cocaine Users

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Abstract: *Objectives:* Stimulant use is associated with increased anxiety and a single administration of dexamphetamine increases amygdala activation to biologically salient stimuli in healthy individuals. Here, we investigate how current cocaine use affects amygdala activity and amygdala connectivity with the prefrontal cortex in response to biologically salient stimuli in an emotional face matching task (EFMT). *Experimental design:* Amygdala activity and amygdala connectivity during the EFMT were assessed in 51 cocaine using males and 32 non-drug-using healthy males using functional magnetic resonance imaging (fMRI). Within the cocaine use group, we explored whether amygdala activation was associated with age of first use of cocaine and duration of cocaine use to distinguish between amygdala activation alterations as a cause or a consequence of cocaine use. *Principal observations:* We observed hyperactivity of the amygdala, thalamus, and hippocampus and reduced amygdala connectivity with the anterior cingulate gyrus in response to angry and fearful facial expressions in current cocaine users compared to controls. Increased amygdala activation was independently associated with earlier age of first cocaine use and with longer exposure to cocaine. *Conclusions:* Our findings

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suggest that amygdala hyperactivity to biologically salient stimuli may represent a risk factor for an early onset of cocaine use and that prolonged cocaine use may further sensitize amygdala activation. High amygdala activation to emotional face processing in current cocaine users may result from low prefrontal control of the amygdala response to such stimuli. *Hum Brain Mapp 36:4222–4230, 2015.*

Key words: cocaine dependence; amygdala; drug abuse; emotion; magnetic resonance imaging; fMRI

INTRODUCTION

The amygdala is a brain structure involved in emotional responses and fear recognition [Morris et al., 1996; Whalen et al., 1998]. While fear recognition is impaired in cocaine users [Kemmis et al., 2007; Morgan and Marshall, 2013], studies investigating the relation between cocaine use and amygdala function in response to emotional stimuli are lacking. Stimulants can induce fear and anxiety and withdrawal from repeated cocaine administration leads to increased anxiety responses [Angrist and Gershon, 1970; Blanchard and Blanchard, 1999; Hall et al., 1988; Murphy et al., 2001]. In healthy individuals, a single administration of the stimulant dextroamphetamine resulted in elevated amygdala activation while viewing angry and fearful facial expressions [Hariri et al., 2002]. During a reward and loss processing task, Patel et al. [2013] observed reduced amygdala activation during loss prospect and proposes that this reflects emotional/motivational abnormalities in cocaine users, thus referring to a pre-existent problem. In contrast, in abstinent methamphetamine-dependent patients (abstinent between 5 and 30 days), amygdala activation upon emotional stimuli was similar to non-drug using control subjects [Kim et al., 2011; Payer et al., 2008]. This latter finding suggests that either the effects of repeated stimulant use on the amygdala are reversible or that chronic stimulant use has no effect on amygdala activation in humans.

Thus far, no data are available on amygdala activation to emotional stimuli in active cocaine users. Amphetamines and cocaine are strong stimulant drugs that affect brain dopamine, serotonin, and norepinephrine systems, however, by different mechanisms of action: by blocking reuptake (cocaine) and by promoting monoamine release (amphetamine) [Cagniard et al., 2014; Silvia et al., 1997]. As such, observed findings from methamphetamine studies cannot directly be extrapolated to cocaine users. Furthermore, it is not clear whether possible differences in amygdala activation are a cause or a consequence of cocaine use, because there is no information on the relation between amygdala activation and age of first use of cocaine and duration of cocaine use.

The amygdala is part of a larger emotion circuitry that includes the anterior cingulate cortex (ACC). The ACC is part of the medial prefrontal cortex and plays an important role in emotion-based decision making. The ACC is known to be hypoactive in cocaine users during emotionally salient tasks, and this effect appears to be proportional to drug use severity [Goldstein et al., 2009]. Amygdala connections to (pre)frontal brain regions have been described earlier [Davis and Whalen, 2002; Price, 2003] and relate to the suppression of a response to negative emotions and seem to be instrumental in the choice of appropriate behaviors [Quirk and Beer, 2006]. Moreover, prenatal cocaine exposure has been shown to alter amygdala activity and its functional and structural connectivity with prefrontal brain regions [Li et al., 2013]. Reduced connectivity between the amygdala and ACC in cocaine users could represent reduced capacity to choose appropriate behavior upon biologically salient stimuli. However, it is unknown whether functional connectivity between the amygdala and the ACC is affected in current cocaine users.

Here, we compare amygdala activation to fearful and angry faces and amygdala-ACC connectivity between current cocaine users and non-drug using healthy controls. We hypothesize that cocaine users show higher amygdala activation during an emotional face matching task (EFMT) and reduced connectivity between the amygdala and ACC. In addition, we explore the association between amygdala activation with age of first use of cocaine and with the duration of cocaine use in current cocaine users.

METHODS AND MATERIALS

Subjects

Male healthy non-drug using controls (HC) and male current cocaine users (COC) of 22–50 years old were recruited through local advertisement. Cocaine users were included when using at least 1 g of cocaine during at least two occasions per week for the last six consecutive months prior to inclusion. With the exception of heroin, other drug use was allowed. Cocaine users were instructed not to use any drug (except nicotine) at least 10 h before scanning. Cocaine has a half-life of only 1 h, and no acute effects of cocaine are expected 10 h after last consumption. HCs were non-drug users (with the exception of alcohol use) and were instructed not to use alcohol at least 10 h before scanning. Participants were excluded if having a psychotic or bipolar disorder, currently taking medication, if ever being treated for a prior psychiatric or neurological disorder, if having prior head trauma, or if having metal in their body.

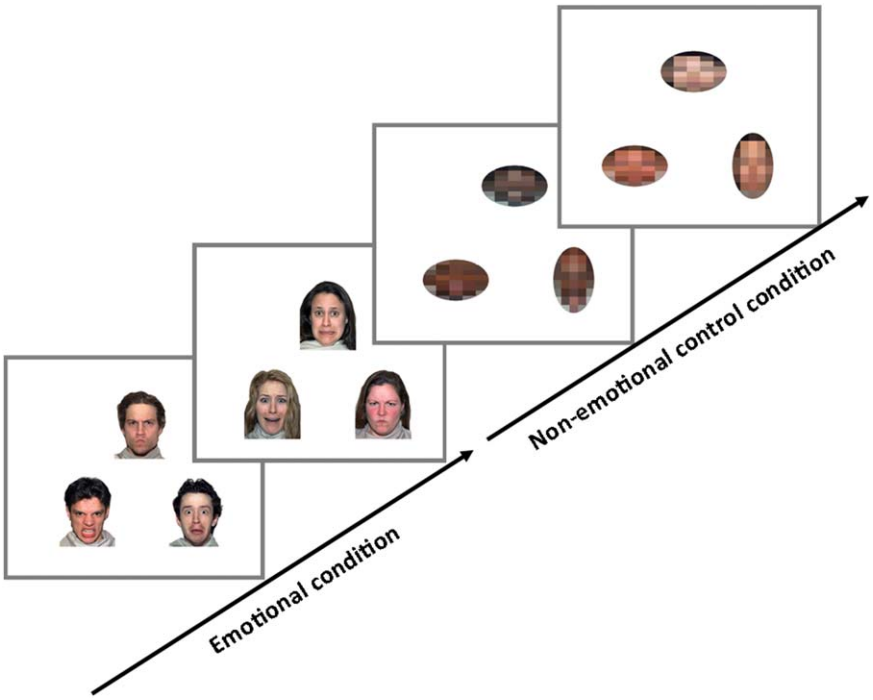

Figure 1.
The EFMT including blocks of an emotional (faces) condition interleaved with blocks of a non-emotional control condition (ellipses). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The study was approved by the Ethical Review Board of the Academic Medical Center of the University of Amsterdam, The Netherlands. Participants gave written informed consent.

Clinical Assessments

Psychiatric disorders were assessed using the Mini International Neuropsychiatric Interview [MINI; Sheehan et al., 1998]. Premorbid intellectual functioning (IQ) was estimated using the Dutch version of the National Adult Reading Test [DART; Schmand et al., 1991]. Smoking behavior and nicotine dependence were assessed using the Fagerström Test for Nicotine Dependence [FTND; Heatherton et al., 1991]. Drug- and alcohol use was assessed during a personal interview with separate questions about recreational and regular drug and alcohol use, detailed by dose, time, and frequency of use. Depressive symptoms and individual levels of impulsivity were assessed using the Beck Depression Inventory [BDI; Beck and Steer, 1987] and the Barratt Impulsivity Scale [BIS; Patton et al., 1995], respectively.

Magnetic Resonance Imaging

Data were collected on a 3.0-T Intera full-body scanner (Philips Medical Systems, Best, The Netherlands) using a 32-channel SENSE head coil. Echo planar images (EPIs) were acquired covering the whole brain, with a total of 37 ascending axial slices (3 mm $\times$ 3 mm $\times$ 3 mm voxel size; slice gap 3 mm; Repetition Time (TR)/Echo Time (TE) 2,000 ms/28 ms; matrix 80 $\times$ 80). A T1-3D high resolution anatomical scan (TR/TE 8.3/3.8; matrix 240 $\times$ 187; 1 $\times$ 1 $\times$ 1 voxel; transverse slices) was acquired for coregistration with the T1-weighted EPIs. None of the subjects showed head movement larger than the voxel size (<3 mm) in any direction. Mean and standard deviation of motion during scanning were 0.7 mm $\pm$ 0.4 mm in any direction and 0.2 $\pm$ 0.1 mm from scan-to-scan.

Emotional Face Matching Task

The EFMT [adapted from Hariri et al., 2002; van Marle et al., 2011] was used, including two blocks of an emotional (faces) condition interleaved with three blocks of a non-emotional control condition (ellipses) (see Fig. 1). The emotional condition consisted of three faces projected on the screen, from which participants had to select one of two faces (bottom row) that corresponded to the emotion presented on the face in the upper row. The presented faces were from different individuals and represented angry or fearful male and female individuals, presented for 5 s each [Hariri et al., 2002]. The non-emotional control condition presented ellipses depicted horizontally or vertically, and participants were again required to select one of

two ellipses presented horizontally or vertically (bottom row) to match the ellipse presented in the upper row [Hariri et al., 2002]. Each block consisted of six trials each, resulting in 30 s blocks. The total task duration was 2.5 min.

Task stimuli were generated with E-Prime and projected on a 61 cm × 36 cm projection screen. Task stimuli were visible through a mirror (17 cm × 10 cm) fixated on the head-coil (distance mirror-eyes 8 cm; distance projection screen-mirror: 113 cm). Participants responded by pressing a button on right and left button boxes (4-button box HHSC-2x4-C). The head was stabilized with foam for minimal head movement.

Behavioral Data Analysis

Normality of all data was assessed with Shapiro–Wilk tests. Differences between groups in clinical assessments (IQ, age, FTND scores, BDI, BIS, proportion of alcohol, nicotine and illicit drug users) were analyzed using independent sample *t*-tests, non-parametric Mann–Whitney *U* tests, or Chi-square tests where appropriate.

Performance on the EFMT was analyzed using independent sample *t*-test (for between group effects) and paired sample *t*-tests (for between condition effects). Non-parametric Mann–Whitney *U* tests were used for accuracy data. Data were analyzed using SPSS version 20 (Statistical Package for the Social Sciences) and are presented as mean ± standard deviation (SD) for normally distributed data and as median ± interquartile range (IQR) for non-parametric data. *P*-values <0.05 were considered significant.

fMRI Data Analysis

Image analysis was performed in SPM8 (Statistical Parametric Mapping; Welcome Department of Cognitive Neurology, London UK). EPIs were slice-time corrected, realigned to correct for head movement, co-registered with the individual anatomical scan, normalized to Montreal Neurological Institute (MNI) space, and spatially smoothed with a 3-D Gaussian kernel of 8 mm at FWHM. Statistical analysis was performed within the framework of the general linear model. The first-level analysis included the two experimental conditions modeled as box-car regressors convolved with the canonical hemodynamic response function of SPM8, the realignment parameters to model potential movement artifacts, and high-pass filtering (cut-off 1/128 Hz). Contrast images comparing the emotional face and non-emotional control conditions were obtained, and analyzed in random effects models.

For the neural responsivity analyses, the main effects of task were analyzed using one sample *t*-tests. Group differences were analyzed using ANCOVA with BDI and BIS, two features with inherent differences between drug users and HCs, as covariates of no interest. Statistical tests were family-wise error (FWE) rate corrected for multiple comparisons. Because of our *a priori* hypothesis about the amygdala, we corrected the statistical tests for the amygdala on the voxel-level for the volume of the amygdala that was anatomically defined [Maldjan et al., 2003; Tzourio-Mazoyer et al., 2002; Worsley et al., 1996]. For the rest of the brain, we corrected the statistical tests on the cluster-level ($P < 0.05$) across the whole brain [Friston et al., 1994]. Clusters were defined using an initial height threshold of $P < 0.001$ uncorrected.

For the functional connectivity analyses, the time-course of amygdala activity was extracted in a sphere with a 5 mm radius around the peak activation in the amygdala region identified from the functional imaging analysis ($x = -28$, $y = -8$, $z = -12$). To obtain time-course correlation images irrespective of the experimental conditions, a new statistical model was constructed with the amygdala time-course as covariate of interest, and the convolved box-car regressors for the experimental conditions and realignment parameters as covariates of no interest. Time course correlation images were obtained and entered into subsequent random-effects analyses. ANCOVAs were used to identify brain regions with a correlated time-course that differed between groups of cocaine users and healthy controls. Because of our *a priori* hypothesis regarding the ACC, we corrected the statistical tests for the ACC on the voxel-level for volumes that were based on a previous study that showed reduced ACC activity in cocaine users during an EFMT [$x = -9$, $y = 48$, $z = -12$, and $x = 6$, $y = 0$, $z = 45$; Goldstein et al., 2009]. For the rest of the brain, we corrected the statistical tests on the cluster-level ($P < 0.05$) across the whole brain [Friston et al., 1994]. Clusters were defined using an initial height threshold of $P < 0.001$ (uncorrected).

For correlation analyses, we extracted data from the anatomically defined amygdala. We analyzed effects of age of first cocaine use and duration of cocaine use on amygdala activity using multiple linear regression in SPSS. Amygdala activation (left or right) was entered as the dependent variable, BDI and BIS scores were entered as covariates, and age of first use of cocaine and duration of cocaine use were entered as independent variables. Additionally, to investigate a possible interaction between age of first cocaine use and duration of cocaine use, the interaction term "duration of cocaine use × age of first use of cocaine" was entered into the model. Upon non-significance of the interaction term, this term was removed and a model with only main effects of age of first use, years of use, and the covariates BDI and BIS was fitted. The strength of the association between the variables of interest and the outcomes was reported using partial correlation coefficients and the *P*-values.

RESULTS

A total of 33 HCs and 55 COCs were recruited and all participated. Five participants (1 HC and 4 COCs) had

TABLE I. Clinical characteristics of cocaine dependent individuals (COC) and non-drug using healthy controls (HC)

	COC ($n = 51$)	HC ($n = 32$)	P value
Age (years)	32 ± 8	33 ± 9	0.410
IQ	102 ± 9	105 ± 10	0.084
BDI	9.1 ± 5.6	3.3 ± 3.5	< 0.001
BIS total	72.2 ± 9.7	59.8 ± 8.2	< 0.001
Cocaine use			
Users (%)	100%	0%	
Age of first use	20.3 ± 5.2	—	
Years of regular use	7.5 ± 5.3	—	
Grams per session	1.2 ± 0.8	—	
Days of use last month	11.3 ± 6.8	—	
Route of administration			
Smoking (%)	9.4%	—	
Snorting (%)	90.6%	—	
Nicotine use		—	
Smokers (%)	73.6%	0%	
FTND	5.1 ± 2.1	—	
Alcohol use			
Drinkers (%)	96.2%	86.7%	0.106
Units per week (10 g units)	19 ± 18	12 ± 11	0.084
Cannabis use			
Users (%)	46.2%	0%	
Use per week (g)	4.2 ± 5.1	—	
Ecstasy use			
Users (%)	38.5%	0%	
Use in last 6 months (pills)	8.3 ± 8.0	—	
Speed use			
Amount of users (%)	26.9%	0%	
Use in last 6 months (g)	3.4 ± 5.5	—	
Sedative use			
Amount of users (%)	13.5%	0%	
Use in last 6 months (pills)	24.2 ± 29.8	—	
Comorbid psychiatric disorders			
Depression (%)	3.9%	0%	
Dysphoric (%)	0%	0%	
Manic (%)	0%	0%	
Panic (%)	0%	0%	
PTSD (%)	0%	0%	
Obsessive compulsive (%)	0%	0%	
Antisocial personality disorder (%)	21.6%	0%	
Adult ADHD (%)	3.9%	0%	

FTND, Fagerström test of nicotine dependence; BDI, Beck Depression Inventory; BIS, Barratt Impulsivity Scale; PTSD, post-traumatic stress disorder; ADHD, Attention Deficit/Hyperactivity Disorder. Data are presented as means ± standard deviation or as median ± interquartile range (for BDI scores). A P-value < 0.05 is considered statistically significant

many more (>66%) omissions (no response) during the EFMT than all other participants and were excluded from all further analyses. Analyses were thus performed on data from 32 HCs and 51 COCs.

Clinical Characteristics

With the exception of self-reported depression scores (BDI), all data were normally distributed (all Shapiro–Wilk $P > 0.204$; NS). Groups did not differ in age and premorbid intellectual functioning. However, there was a significant difference between groups on BDI depression scores and on total BIS trait impulsivity scores (Table I). Table I also shows that the vast majority of the COC group snorted cocaine and that there was extensive polydrug use in this group, including the use of tobacco, cannabis, ecstasy, speed, and sedatives. In the COC group, 82% of included participants met the diagnosis of DSM-IV cocaine dependence: 14 (27%) cocaine abuse and 28 (55%) cocaine dependence. Comorbid psychiatric disorders are also presented in Table I.

TABLE II. Performance data of the emotional processing task for cocaine dependent individuals (COC) and non-drug using healthy controls (HC)

Emotional faces task performance	COC ($n = 51$)	HC ($n = 32$)	P value
RT emotional (s)	2.15 ± 0.49	2.16 ± 0.63	0.916
RT control (s)	1.29 ± 0.54	1.22 ± 0.58	0.590
Accuracy emotional (%)	100.00 ± 8.33	100.00 ± 14.60	0.809
Accuracy control (%)	94.44 ± 11.11	100.00 ± 5.60	0.645

RT, reaction times. Data are presented as mean ± standard deviation (RT) and as median ± interquartile range (Accuracy). A P value < 0.05 indicates statistical significance

Emotional Faces Task

Behavioral data

Reaction times (RTs) were normally distributed (Shapiro–Wilk $P > 0.071$), but accuracy was not normally distributed (Shapiro–Wilk $P < 0.001$). RTs on emotional faces and geometrical control stimuli were similar for both groups (see Table II). Also, no between group differences were observed in accuracy on the EFMT or on the control task, suggesting that conditions were similar in difficulty for both groups.

fMRI task activation

Consistent with previous findings [Hariri et al., 2002; Kim et al., 2011], a significant main effect of task (faces > ellipse stimuli) was present in a cluster (coordinates: $x = 42$, $y = -52$, $z = -18$; voxel size = 45,522: $Z > 8$; P(FWE) < 0.001) encompassing the right and left fusiform gyrus (coordinates $x = 42$, $y = -52$, $z = -18$ and $x = -40$, $y = -46$, $z = -20$), the left and the right amygdala (coordinates $x = 23$, $y = -8$, $z = -12$ and $x = -20$, $y = -8$, $z = -12$), the right calcarine

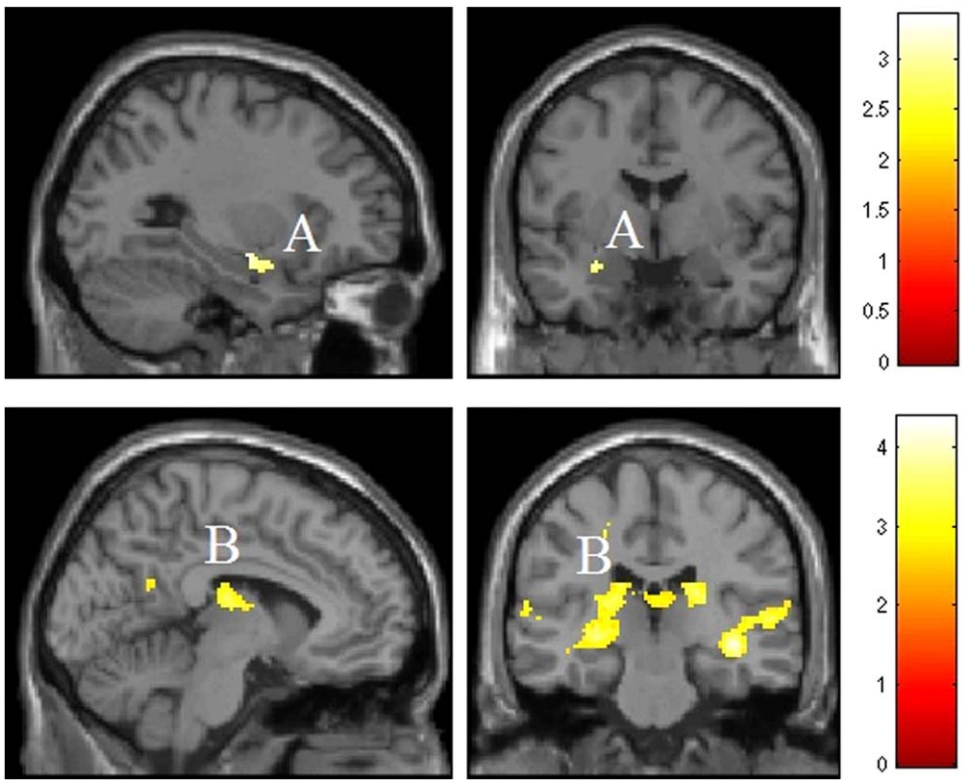

Figure 2.
Cocaine-dependent individuals showing significantly increased activation during an EFMT in the left amygdala (**A**) and in the hippocampus and thalamus region (**B**). Results are depicted with a threshold of $P < 0.005$ uncorrected to show the extent of activation. The color bar represents the corresponding T values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

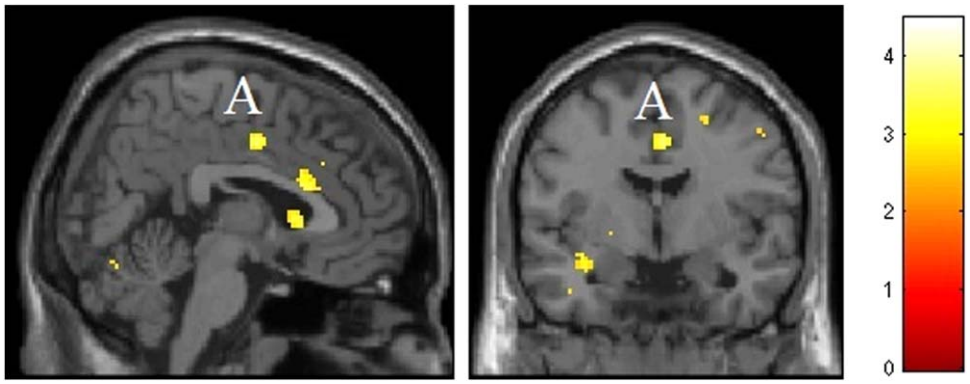

Figure 3.
Cocaine-dependent individuals show significantly reduced functional connectivity between the amygdala and anterior cingulate gyrus (**A**). Results are depicted with a threshold of $P < 0.005$ uncorrected to show the extent of activation. The color bar represents the corresponding *T* values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

sulcus (coordinates $x = 26$, $y = -96$, $z = 0$), and the right and left inferior occipital cortex (coordinates $x = 40$, $y = -82$, $z = -6$ and $x = -30$, $y = -92$, $z = -8$). A second cluster (coordinates: $x = -6$, $y = 18$, $z = 50$; voxel size = 2,210: $Z = 7.44$; $P(\text{FWE}) < 0.001$) showed activation in the left and right supplementary motor area (SMA) (coordinates $x = -6$, $y = 18$, $z = 50$ and $x = 6$, $y = 18$, $z = 50$) and in the right and left medial superior frontal gyrus (coordinates $x = 6$, $y = 44$, $z = 42$ and $x = -4$, $y = 54$, $z = 34$). Reduced brain activation (ellipse > face stimuli) was observed in the right middle cingulate gyrus (coordinates: $x = 2$, $y = -28$, $z = 44$; voxel size = 2,704: $Z = 7.49$; $P(\text{FWE}) < 0.001$), in the right anterior cingulate gyrus (coordinates: $x = -2$, $y = -40$, $z = -2$; voxel size = 4,863: $Z = 6.34$; $P(\text{FWE}) < 0.001$), in the right supramarginal gyrus (coordinates: $x = 60$, $y = -46$, $z = 44$; voxel size = 3,493: $Z = 6.86$; $P(\text{FWE}) < 0.001$), and in the left supramarginal gyrus (coordinates: $x = -62$, $y = -30$, $z = 38$; voxel size = 2,198: $Z = 5.41$; $P(\text{FWE}) < 0.001$).

fMRI group differences

There was significantly higher activity during the face matching condition than during the control condition in the COC group compared to the HC group in the left amygdala (coordinates: $x = -28$, $y = -8$, $z = -12$; $Z = 3.30$; $P_{(\text{SVC})} = 0.016$) (Fig. 2) and in a cluster including the left hippocampus and the left thalamus (coordinates: $x = -20$, $y = -26$, $z = 14$; voxel size = 520: $Z = 4.10$; $P_{(\text{FWE})} < 0.001$). No regions with lower activation in COC compared to HCs were observed.

Functional connectivity analyses

The results showed reduced connectivity between the left amygdala and the left caudal dorsal ACC (coordinates: $x = 4$, $y = -4$, $z = 42$; voxel size = 11: $Z = 3.74$; $P_{(\text{SVC})} = 0.015$; see Fig. 3) in COCs compared to HCs. Overall, this ACC subregion was positively coupled to the amygdala ($P < 0.001$; $Z > 8$), which implies that coupling between the amygdala and the ACC was relatively reduced.

Correlation analyses

To study the effect of age of first cocaine use and duration of cocaine use on amygdala activation, a multiple linear regression analysis was performed for the COC group. The analysis was adjusted for the known effects of BDI and BIS. All data were normally distributed (Shapiro–Wilk all $P > 0.130$). First, we tested whether the effects of years of use was influenced by the age of first use of cocaine. Regression analysis showed no significant interaction between these two variables (left amygdala $P = 0.924$; right amygdala $P = 0.799$), indicating that the effect of the duration of cocaine use is not influenced by the age of first use of cocaine and vice versa.

The unique effects of the independent variables "age of first cocaine use" and "duration of cocaine use" on left and right amygdala activation were estimated using multiple linear regression, whereby the effect of one independent variable was corrected for the effect of the other independent variable and for the covariates BDI and BIS. Age of first cocaine use was negatively associated with amygdala activation (left amygdala: $r = -0.36$, $P = 0.012$; right amygdala $r = -0.32$, $P = 0.026$), i.e., individuals with a relatively early age of first use of cocaine showed higher amygdala activation. Years of cocaine use was positively associated with amygdala activation (left amygdala: $r = 0.36$, $P = 0.013$; right amygdala: $r = 0.32$, $P = 0.028$), i.e., individuals with a longer duration of cocaine use showed higher amygdala activation.

Sensitivity analyses

In an additional sensitivity analysis, we included comorbid drug use (i.e., use of nicotine, alcohol, cannabis,

ecstasy, speed, and sedative use) as additional covariates in the model to assess whether correlations between age of first use of cocaine and duration of cocaine use and amygdala activation would remain statistically significant. When entering the additional covariates into the model, none of these covariates were found to be significantly associated with amygdala activation (all $P > 0.245$), left and right amygdala activation remained significantly correlated with years of use (left amygdala: $r = 0.49$, $P = 0.02$; right amygdala: $r = 0.39$, $P = 0.013$), and a clear trend remained for the correlation between age of first use of cocaine and amygdala activation (left amygdala: $r = 0.29$, $P = 0.073$; right amygdala: $r = 0.29$, $P = 0.068$).

DISCUSSION

The current study shows significant hyperactivity of the amygdala and reduced connectivity between the amygdala and the ACC in response to angry and fearful emotional facial expressions in current cocaine users compared to non-drug-using controls. Current cocaine users with a relatively early age of first use of cocaine or a longer duration of cocaine use show higher amygdala activation than those with a relatively late age of first use of cocaine or a shorter duration of cocaine use.

The present finding that cocaine-dependent individuals display increased amygdala activation during the EFMT is in correspondence with the statement by Patel et al. [2013] that cocaine users show emotional abnormalities, based on their findings of altered (however reduced) amygdala activation during the prospect of loss [Patel et al., 2013]. The differences regarding hypo- vs. hyperactivation of the amygdala in cocaine users between our study and the study by Patel et al., [2013] may be due to differences in the tasks used; a monetary incentive delay task addresses reward and loss prospect, anticipation, and outcome [Patel et al., 2013], while the EFMT as used in this study focuses on emotional processing without a reward or loss component. Also, our findings differ from the observation that abstinent methamphetamine-dependent patients do not show amygdala hyperactivity compared to non-stimulant users [Kim et al., 2011; Payer et al., 2008]. This could be due to inherent differences between cocaine and amphetamines regarding half-life, working mechanism, and involved neurotransmitter systems, but could also be due to the studied population: the studies in methamphetamine-dependent individuals were performed in abstinent treatment-seeking stimulant users (with abstinence ranging between 5 and 30 days), whereas our sample consists of non-treatment-seeking current cocaine users who were abstinent only for about 10 h.

Functional connectivity between the amygdala and prefrontal cortex as well as amygdala activation during the EFMT has not been studied previously in current cocaine users. The prefrontal cortex is thought to be involved in the suppression of a response to negative emotions and seems to be instrumental in the choice of appropriate behaviors [Quirk and Beer, 2006]. Altered amygdala–prefrontal cortex connectivity may be of crucial importance in both the initiation and continuation of cocaine use. In this respect, the reduced connectivity between the amygdala and the ACC in COCs compared to HCs may represent a reduced capacity to regulate the amygdala in the presence of biologically salient (negative) stimuli, including drug-related stimuli and related processes of reward and relief craving. Also, our findings imply that the amygdala is dysfunctional in current cocaine users. More specifically, the positive association of amygdala hyperactivity in response to biologically salient stimuli in the EFMT with age of first use of cocaine and with duration of cocaine use proposes that amygdala hyperactivity may be both cause and consequence of cocaine use.

This study is the first to investigate amygdala activation during an EFMT in a large sample of current cocaine users. Previous studies have shown amygdala neuronal firing upon cocaine administration in drug dependent animals [Carelli et al., 2003]. Yet, this was never translated to amygdala activity and activation during an EFMT in humans, for which this study provides the first evidence. One limitation of this study is its cross-sectional design, which limits causal inference and the direction of causality. However, longitudinal and randomized studies on the development of drug addiction are non-existing for reasons of logistics and ethics. Nevertheless, analyses that simultaneously take into account the effects of several time-related variables (years of drug use; duration of use) can dissect the relationships of these time-dependent variables on the outcome (amygdala hyperactivity). This approach allows us to make a tentative distinction between the causal and consequential effects of drug use. Another limitation is that connectivity analyses do not show the direction of the connectivity (to or from) the ACC, which should be investigated in future studies. For future studies, we also propose to include a group of non-cocaine drug users and a group of cocaine abusers in remission. Finally, most current cocaine users were poly-drug users and it cannot be excluded that part of the observed relations between brain activation and cocaine use are due to the use of nicotine, alcohol, or illicit drugs other than cocaine. However, cocaine was the primary substance of abuse in all subjects and our findings were very similar after we controlled for the use of other drugs.

CONCLUSIONS

In summary, this is the first study showing increased amygdala activation during an EFMT and decreased connectivity between the amygdala and the ACC in current cocaine users compared to non-drug-using healthy controls. Moreover, amygdala hyperactivity was independently associated with early age of first use of cocaine and longer duration of cocaine use, suggesting that high

amygdala activation to emotional stimuli is both a risk factor for the early onset of cocaine use and a consequence of prolonged cocaine use.

REFERENCES

Angrist BM, Gershon S (1970): The phenomenology of experimentally induced amphetamine psychosis-preliminary observations. Biol Psychiatry 2:95–107.

Beck AT and Steer RA (1987). Manual for Revised Beck Depression Inventory. San Antonio, TX: Psychological Corporation.

Blanchard DC, Blanchard RJ (1999): Cocaine potentiates defensive behaviors related to fear and anxiety. Neurosci Biobehav Rev 23:981–991.

Cagniard B, Sotnikova TD, Gainetdinov RR, Zhuang X: The dopamine transporter expression level differentially affects responses to cocaine and amphetamine. J Neurogenet 28:112–121.

Carelli RM, Williams JG, Hollander JA (2003): Basolateral amygdala neurons encode cocaine self-administration and cocaine-associated cues. J Neurosci 23:8204–8211.

Davis M, Whalen PJ (2002): The amygdala: Vigilance and emotion. Mol Psychiatry 6:13–34.

Friston KJ, Worsley KJ, Frackowiak RS, Mazziotta JC, Evans AC (1994): Assessing the significance of focal activations using their spatial extent. Hum Brain Mapp 1:210–220.

Goldstein RZ, Alia-Klein N, Tomasi D, Carrillo JH, Maloney, T, Woicik PA, Wang R, Telang F, Volkow ND (2009): Anterior cingulate cortex hypoactivations to an emotionally salient task in cocaine addiction. Proc Natl Acad Sci USA 106:9453–9458.

Hall RC, Popkin MK, Beresford TP, Hall AK (1988): Amphetamine psychosis: Clinical presentations and differential diagnosis. Psychiatr Med 6:73–79.

Hariri AR, Mattay VS, Tessitore A, Fera F, Smith WG, Weinberger DR (2002): Dextroamphetamine modulates the response of the human amygdala. Neuropsychopharmacology 27:1036–1040.

Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO (1991): The fagerström test for nicotine dependence: A revision of the fagerström tolerance questionnaire. Br J Addict 86:1119–1127.

Kemmis L, Hall JK, Kingston R, Morgan MJ (2007): Impaired fear recognition in regular recreational cocaine users. Psychopharmacology (Berl) 194:151–159.

Kim YT, Song HJ, Seo JH, Lee JJ, Lee, J, Kwon DH, et al. (2011): The differences in neural network activity between methamphetamine abusers and healthy subjects performing an emotion-matching task: Functional MRI study. NMR Biomed 24:1392–1400.

Li Z, Santhanam P, Coles CD, Ellen Lynch M, Hamann S, Peltier S, Hu X (2013): Prenatal cocaine exposure alters functional activation in the ventral prefrontal cortex and its structural connectivity with the amygdala. Psychiatry Res 213:47–55.

Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003): An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19:1233–1239.

Morgan MJ, Marshall JP (2013): Deficient fear recognition in regular cocaine users is not attributable to elevated impulsivity or conduct disorder prior to cocaine use. J Psychopharmacol 27: 526–532.

Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, Dolan RJ (1996): A differential response in the human amygdala to fearful and happy facial expressions. Nature 383:812–815.

Murphy CA, Heidbreder C, Feldon J (2001): Acute withdrawal from repeated cocaine treatment enhances latent inhibition of a conditioned fear response. Behav Pharmacol 12:13–23.

Patel KT, Stevens MC, Meda SA, Muska C, Thomas AD, Potenza MN, Pearlson GD (2013): Robust changes in reward circuitry during reward loss in current and former cocaine users during performance of a monetary incentive delay task. Biol Psychiatry 74:529–537.

Patton JH, Stanford MS, Barratt ES (1995): Factor structure of the Barratt impulsiveness scale. J Clin Psychol 51:768–774.

Payer DE, Lieberman MD, Monterosso JR, Xu J, Fong TW, London ED (2008): Differences in cortical activity between methamphetamine-dependent and healthy individuals performing a facial affect matching task. Drug Alcohol Depend 93:93–102.

Price JL (2003): Comparative aspects of amygdala connectivity. Ann NY Acad Sci 985:50–58.

Quirk GJ, Beer JS (2006): Prefrontal involvement in the regulation of emotion: Convergence of rat and human studies. Curr Opin Neurobiol 16:723–727.

Schmand B, Bakker D, Saan R, Louman J (1991): The Dutch reading test for adults: A measure of premorbid intelligence level. Tijdschr Gerontol Geriatr 22:15–19.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs, J, Weiller E, et al. (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59:22–33.

Silvia CP, Jaber M, King GR, Ellinwood EH, Caron MG (1997): Cocaine and amphetamine elicit differential effects in rats with a unilateral injection of dopamine transporter antisense oligodeoxynucleotides. Neuroscience 76:737–747.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard, O, Delcroix N, et al. (2002): Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15:273–289.

van Marle HJ, Tendolkar I, Urner M, Verkes RJ, Fernández G, van Wingen G (2011): Subchronic duloxetine administration alters the extended amygdala circuitry in healthy individuals. Neuroimage 55:825–831.

Whalen PJ, Rauch SL, Etcoff NL, McInerney SC, Lee MB, Jenike MA (1998): Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci 18:411–418.

Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC (1996): A unified statistical approach for determining significant signals in images of cerebral activation. Hum Brain Mapp 4:58–73.