# Sex-dependent differences in the neural correlates of cocaine and emotional cue-reactivity in regular cocaine users and non-drug using controls: understanding the role of duration and severity of use

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**Abstract**

Cocaine use disorder (CUD) is becoming more prevalent in females, but research into sex-dependent neural mechanisms underlying cocaine use is lacking. Accordingly, the main objective of the current study was to investigate sex-dependent differences in the neural correlates of cocaine and emotional cue reactivity within regular cocaine users (CUs) and non-cocaine-using controls (non-CUs). A cocaine and emotional cue-reactivity fMRI paradigm was completed by 31 male and 26 female CUs and 28 male and 26 female non-CUs. A region of interest analysis was performed to test for sex-specific differences in cocaine and emotional cue-induce activation of the dorsal striatum (DS), ventral striatum (VS), amygdala, and dorsal anterior cingulate cortex (dACC). Although there were no significant sex-dependent differences between CUs and non-CUs in neural cocaine and emotional cue reactivity, exploratory analyses demonstrated that the association between cocaine cue-induced activation of the DS and amygdala and cocaine use severity was significantly moderated by sex: while this association was positive female CUs, it was negative in male CUs. Similarly, the relationship between emotional cue-induced activation of the dACC and VS and years of regular cocaine use was also moderated by sex: while this association was negative in female CUs, it was positive in male CUs. While exploratory, the current findings highlight the importance of taking into account sex differences when studying the underlying mechanism of CUD, as this may pave the way for the identification of sex-specific treatment targets.

## 1. INTRODUCTION

Cocaine is one of the most commonly used illicit substances around the world and causes severe economic, psychosocial, and psychiatric consequences(Mustaquim *et al.* , 2021). Generally, cocaine use disorder (CUD) is more prevalent in males than females, but this gap is slowly closing due to increased cocaine use in females from 2010 to 2019(Mustaquim *et al.* , 2021). Furthermore, there are several indications for sex differences in clinical profiles of CUD with significant implications for treatment(Fonseca *et al.* , 2021). For instance, in female cocaine users (CUs), there are higher rates of comorbid psychiatric mood disorders(Griffin *et al.* , 1989; Rounsaville *et al.* , 1991) whereas male CUs more often have comorbid alcohol abuse, attention deficit hyperactivity disorder and/or antisocial personality disorder (Griffin *et al.* , 1989; Rounsaville *et al.* , 1991). Accordingly, researchers have begun to explore the various mechanisms underlying sex differences in the development of substance use disorders (SUDs), including CUD(Fonseca*et al.* , 2021).

One of the most frequently applied methods for understanding the underlying neurobiological mechanisms in SUDs are cue reactivity paradigms in which subjects are exposed to substance-related and/or emotional cues (e.g., pictures or videos) in addition to neutral cues during functional magnetic resonance imaging (fMRI) in order to identify brain regions that mediate the development, persistence and treatment of SUD(Courtney *et al.* , 2015). The number of neuroimaging cue reactivity studies has increased considerably during the last several decades according to a recent consensus paper (370 published studies through April 30, 2021)(Ekhtiari

*et al.* , 2022). Yet, one recent systematic review identified only six neuroimaging studies to date that statistically evaluated sex differences in these neurobiological biomarkers in SUD(Nicolas *et al.* , 2022), despite the ubiquitous view that neurobiological sex differences in disease aetiology and neuropathology exist(Fonseca *et al.* , 2021; Nicolas *et al.* , 2022). Ultimately, this necessitates the need for hypothesis driven neuroimaging research into sex differences, primarily due to its implications in understanding the neurobiology of SUD(Courtney *et al.* , 2015; Fonseca *et al.* , 2021; Nicolas*et al.* , 2022; Orsini *et al.* , 2022) and developing sex-tailored treatment designs.

There is increasing evidence of sex-specific involvement of corticostriatal-limbic brain regions during the processing of cocaine or emotional cues(Li *et al.* , 2005; Volkow *et al.* , 2011; Potenza *et al.* , 2012; Canterberry *et al.* , 2016). More specifically, hyperactivation of the striatum, medial PFC, dorsal ACC and amygdala in response to drug cues have been reported in male CU's, while hypoactivation of these regions is generally found in female CUs (Kilts *et al.* , 2004; Volkow *et al.* , 2011; Potenza *et al.* , 2012; Smith *et al.* , 2023). Similarly, hyperactivation of these prefrontal-limbic regions in response to negative emotional cues have been demonstrated in female CUs, while hypoactivation has been demonstrated in male CUs (Li *et al.* , 2005; Potenza *et al.* , 2012). While not all research findings are consistent (Canterberry*et al.* , 2016; Smith *et al.* , 2023), the overall finding is that male CUs, compared to female CUs demonstrated hyperactivation of the limbic corticostriatal brain regions, including the dorsal (DS) and ventral striatum (VS), dorsal ACC and amygdala, in response to cocaine related cues, while female CUs compared to male CUs demonstrate hyperactivation of these regions in response to negative emotional or stress-related cues(Nicolas *et al.* , 2022).

Nevertheless, results from previous studies should be taken with caution due to numerous identified limitations, including heterogeneity in cue reactivity paradigms (i.e., stress imagery(Li *et al.* , 2005; Potenza *et al.* , 2012), drug/emotional-related pictures(Canterberry *et al.* , 2016; Zhang *et al.* , 2021) or video's(Volkow *et al.* , 2011), uneven male to female ratios(Li*et al.* , 2005; Volkow *et al.* , 2011; Zhang *et al.* , 2021), a lack of control groups(Li *et al.* , 2005; Volkow *et al.* , 2011; Zhang *et al.* , 2021), and small samples sizes(Li*et al.* , 2005; Volkow *et al.* , 2011; Tuit *et al.* , 2013; Canterberry *et al.* , 2016; Kober *et al.* , 2016; Zhang*et al.* , 2021) that are considered well below the recommended number needed for reproducibility of results in fMRI articles(Ekhtiari*et al.* , 2022). Therefore, the main objective of the current study was to investigate sex-dependent differences in the neural correlates of cocaine and (negative) emotional cue reactivity within CUs and non-CUs, within VS, DS, amygdala and dACC. In line with previous research, it was hypothesized that male CUs, compared to female CUs and non-drug using controls, would show stronger activation of the VS, DS, amygdala and dACC in response to cocaine-related cues, whereas female CUs compared to male CUs and non-drug using controls, would show stronger activation of these regions to negative emotional cues . A secondary objective was to investigate whether there was a sex-specific relationship between cocaine use characteristics and emotional and cocaine-cue induced activation of these brain regions. It was hypothesized that cocaine use characteristics (use per month, severity of use, onset age and duration of regular use) were specifically related to cocaine-cue related activation of the VS, DS, amygdala and dACC in male CUs, whereas these characteristics where specifically related to emotional cue-related activation of these regions in female CUs. Lastly, there is increasing evidence that fluctuating sex hormones in natural cycling women and synthetic hormones in women that use hormonal contraceptives, strongly influence processes of positive and negative reinforcement(Voorhees*et al.* , 2012; Kokane & Perrotti, 2020). Therefore several exploratory analyses were performed to investigate the effect of menstrual phase and hormonal contraceptive use on emotional and cocaine cue reactivity of which the methods and results are described in the supplementary material.

## 2. METHODS AND MATERIALS

### 2.1 Participants

The current study is part of larger project aimed to investigate sex differences in the neurocognitive mechanisms underlying CUD. While the main objective of this project was to investigate sex differences in the neural correlates of (negative) emotional and cocaine cue-reactivity, two more exploratory studies to investi-

gate sex differences in the neural correlates of working memory and cortical morphometry of the insula, have already been published elsewhere(Cousijn *et al.* , 2021; Abdel Malek *et al.* , 2022). The total sample (n = 111) aged 18 to 43 years consisted of regular CUs (n = 57, 45.6% females) and matched non-CUs (n = 54, 48.1% females). All participants were free from any MRI contraindications. General exclusion criteria were potential post-traumatic stress disorder (PTSD) ([?] 2 on the Jellinek-PTSD screening questionnaire)(van Dam *et al.* , 2013), an age outside the range of 18 to 45, and contraindications for MRI scanning. For the non-CU group, individuals were excluded if they smoked cigarettes, had a score higher than 12 on the Alcohol Use Disorders Identification Test (AUDIT)(Saunders *et al.* , 1993) or used other drugs or cocaine no more than five times in the past six months or year, respectively. Individuals were included in the CU group when they used cocaine (intranasally) at least four times per month. Participants provided informed consent and received a monetary compensation for participation. The current study was approved by the Ethics Review Board of the Faculty of Social and Behavioral Sciences, University of Amsterdam (ERB number: 2019-DP-9964).

### 2.2 Procedures

All participants were recruited through online and offline advertisements (e.g., social media and poster advertisements) in the area of Amsterdam, the Netherlands. Next, participants were asked to provide online informed consent for an online screening procedure in order to assess in- and exclusion criteria. On the research testing day, participants provided written informed consent, completed all the questionnaires, and received an MRI scan. All participants received instructions to abstain from any drug use 24 hours prior to the MRI scan.

### 2.3 Assessment of substance use, psychological functioning and menstrual phase

Substance use was assessed within all participants. For cocaine use severity and related problems in the past twelve months, the Drug Use Disorder Identification Test for cocaine (DUDIT)(Berman *et al.* , 2005) was used. In addition, the Time Line Follow-Back (TLFB) procedure(Robinson *et al.* , 2014) was completed to determine cocaine use (grams and days per month), alcohol use and cannabis use in the 28 days prior to study participation, as well as alcohol . The age of onset was measured by using an in-house questionnaire. Tobacco use was assessed using an in-house questionnaire (number of smoking days per week and cigarettes per day). Finally, a self-report questionnaire based on the Structured Clinical interview for the DSM (SCID)(First, 2015) was used to assess symptoms for CUD, cannabis use disorder, and alcohol use disorder.

All participants completed several self-report questionnaires in order to assess psychological functioning. Specifically, severity of depressive symptoms was measured using the Beck Depression Inventory (BDI-II)(BECK, 1961), state and trait anxiety was measured by using the State and Trait Anxiety Inventory (STAI)(Bados & Gómez-benito, 2010), attention deficit hyperactivity disorder (ADHD) symptom severity was assessed through the ADHD Rating Scale (ADHD-RS)(Sandra Kooij *et al.* , 2008), childhood trauma through the Childhood Trauma Questionnaire (CTQ)(Bernstein *et al.* , 2003) impulsivity was measured by using the Barratt Impulsiveness Scale (BIS-11)(Patton *et al.* , 1995) and education level was assessed with an in-house questionnaire.

Using an inhouse developed questionnaire, hormonal contraceptive use or menstrual phase was determined. For naturally cycling females, menstrual phase was determined using an in-house developed questionnaire that assessed averaged duration of the menstrual cycle and days since last menstruation. Based on this information, females were grouped as hormonal contraceptive users, being in the luteal phase of the menstrual cycle or being in the follicular phase of the menstrual cycle for exploratory analyses. See supplementary materials for more information.

### 2.4 Cocaine and emotional cue reactivity task

During the functional magnetic resonance imaging (fMRI) scan, participants completed a cocaine and emotional cue-reactivity paradigm that consisted of 30 full color cocaine images and 30 emotionally negative images (see Fig. 1). Both conditions were matched with 30 neutral pictures resulting in a total of 120 images. The

paradigm consisted of 3 cocaine blocks, 3 emotionally negative blocks, and 6 control blocks. Every block contained 10 pictures that were each presented for 2.5 seconds. The order of the blocks was random (see Fig. 1). A fixation cross was shown for another 2.5 seconds before every block and between the different blocks. All pictures were full-color images and were rescaled to a 448 x 336 pixel dimension. The negative valence pictures were selected from the 65 images of the open affective standardized image set (OASIS)(Kurdi *et al.* , 2017) that were rated lowest on the valence scale in both sexes. The neutral images were also selected from the OASIS database, particularly those that were rated neutral on the valence scale in both sexes (i.e., between 3.8 and 4.2). Cocaine pictures were derived from an earlier dataset(Kaag*et al.* , 2018). All neutral images were matched accordingly to the negative images with respect to the scene presented, composition, colors, and sex of the people shown.

Participants were presented with the cue-reactivity paradigm on a screen behind the MRI scanner. They were able to view this screen through a mirror that was placed on the MRI head coil. Participants were asked how they felt to assess affect and how much they craved cocaine before the first block and after every block was presented. These questions were answered through response buttons in which participants had to select a number on a visual analogue scale (VAS) that ranged from 1 ("Sad"/"Not all all") to 9 ("Happy"/"Extremely"). If no response was given within 5 seconds, the task continued. All fMRI scans were conducted between 3PM and 6PM to minimize potential effects of the time of day on craving.

### 2.5 fMRI data acquisition and processing

All images were acquired through a 3.0-T Phillips Achieva DS scanner (Philips Medical Systems, Best, the Netherlands) with a 32-channel head coil. First, a T1-3D anatomical scan (TR/TE 8.2/3.8; matrix 240x240; 1x1x1 mm3 voxel; transverse slices) was taken. During the cue-reactivity task, echo planar images (EPIs) covering the whole brain were taken with a total of 36 ascending axial slices (3x3x3 mm3 voxel size; slice gap 3mm; TR/TE 1.999/28ms; matrix 80x80).

The MRI data was preprocessed using the fMRI prep 1.3.2 pipeline(Esteban*et al.* , 2019). First, the anatomical data was corrected for intensity nonuniformity, skull-stripped, spatially normalized, and segmented into cerebrospinal fluid, white matter, and gray matter. Second, the functional data was corrected for susceptibility distortions by using a deformation field followed by co-registration, motion-correction, and smoothing. Third, an independent component analysis for Automatic Removal of Motion Artifacts (ICA-AROMA)(Pruim*et al.* , 2015) was performed that automatically removed (head) motion artifacts and the data was resampled to standard space.

The current study utilized SPM12 to further analyze the fMRI data (http://www.fil.ion.ucl.ac.uk/spm). First level models included separate regressors for the cocaine clue block, the emotionally negative block, and the two neutral blocks. These regressors were convolved with a canonical hemodynamic response function. A high pass filter (1/128 Hz) was included in the first-level model to correct for low-frequency signal drift. The contrasts for the neutral, emotionally negative and cocaine blocks were subsequently entered in a second level model. Subsequently, the Marsbar toolbox (http://marsbar.sourceforge.net) was used to extract the mean activity for the contrasts for each region of interest (DS, VS, amygdala and dACC). In line with previous research , the VS was defined as the nucleus accumbens from the Harvard-Oxford subcortical structure probability atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html) and the DS was defined as the caudate and putamen from the automated anatomical labeling (AAL) atlas(Tzourio-Mazoyer *et al.* , 2002) minus the VS. The dACC was defined as the higher (z-coordinate$>$ 78) part of the ACC defined by the AAL atlas. Finally, the amygdala mask was derived from the AAL atlas.

### 2.6 Statistical analyses

Potential sex and group differences in demographic and clinical characteristics were analyzed, such as ADHD symptoms, depression, anxiety, childhood trauma, and impulsivity. In case of significant effects for sex, group, or sex by group interactions, these outcome measures were included as confounders in follow-up exploratory analyses. Potential sex and group differences in subjective cue-reactivity (craving and affect) were analyzed using two repeated measures ANOVAs, with cue-type (cocaine vs neutral or emotional versus neutral) as

repeated measures, group and sex as between group variable, and craving and negative affect as outcome variables. With regards to the assessment of affect during the cue reactivity paradigm, the current study asked for the participants rating in affect (a negative value represented a negative affect, a positive value reflects a positive affect). Negative or lower scores represent an increase in negative affect, while increases or positive scores represent an increase in positive affect.

Sex-dependent differences in negative emotional and cocaine cue activation of the ROIs was tested using repeated measures ANOVAs. More specifically, cue type (emotional cues versus neutral cues and cocaine cues versus neutral cues) was included as repeated measures in two separate analyses, group and sex as independent factors, and mean framewise displacement (FD) as covariate of non-interest. In case of significant sex by group by cue-type interaction effects, within group and sex follow-up tests were performed.

In order to investigate the moderating role of cocaine use characteristics on emotional and cocaine cue reactivity, several within CU group repeated measures analyses were performed, with cue type (emotional cues versus neutral cues and cocaine cues versus neutral cues) as repeated measure in two separate analyses, mean activation in the ROIs as dependent variable, sex as between group factor, and FD as covariate of non-interest. The following variables were included as covariates of interest, including years of regular cocaine use, age of onset of regular cocaine use, cocaine use severity (DUDIT-scores), and cocaine use per month in grams. In case of significant sex by covariate interaction effects, within sex follow-up tests were performed.

To test for main and interaction effects of group, sex and cue type outside the predefined ROIs, an exploratory whole-brain analysis was performed, with mean FD values for each subject as covariate of non-interest to account for potential motion differences. Whole brain analyses were family-wise error (FWE) rate corrected on cluster level ($p < .05$), with an initial height threshold on voxel level of $p < .001$.

The methods for analyzing the influence of menstrual phase and the use of hormonal contraceptives is described in the supplementary material.

## 3. RESULTS

### 3.1 Demographic and clinical characteristics

Four values of years of regular use and age of onset were missing. These missing data have been imputed based on the regression between age and years of regular use / onset age of regular use in males and females, separately. The following formula were used for years of regular used: -4,11 + (0,31*age) for males and 10,62 + (0,6*age) for females. For onset age of regular use the following formula were used: 4,11+ (0,69*age) for males and 10,63 + (0,4*age) for females.

The CU and non-CU groups were matched on age. Yet, the CU group reported significantly higher scores on ADHD symptom severity, depressive symptoms, impulsivity, childhood trauma and drinking severity. Moreover, there was a sex by group interaction effect in childhood trauma, depressive symptoms and impulsivity, where female CUs reported significantly higher scores. Within the non-CU group, there were no sex differences in these variables. Accordingly, these clinical characteristics have been included as potential confounders in exploratory analyses of the cocaine and emotional cue-reactivity data. Male and female CUs were well matched on cocaine use severity, cocaine use per month, and years of regular use. However, females reported regular use of cocaine at an earlier age than males. An overview of all demographic and clinical data and statistics are presented in Table 1 and 2.

### 3.2 Cocaine Cue Reactivity

#### *3.2.1 Subjective effects*

Cocaine cue induced craving was significantly moderated by group ($p < .001$, $\eta^2 = .24$) but not by sex. More specifically, cocaine cues significantly increased craving in CUs ($p<.001$, $\eta^2 = 0.46$) but not in non-CUs ($p = .23$, $\eta^2 = .03$) (see Fig. 2A). There were no significant main or interaction effects between group, sex and cue type on cocaine-cue induced negative affect.

*3.2.2 Group and sex differences in cocaine cue reactivity and the relationship with subjective changes in negative affect*

Cocaine cues did not induce any significant activation in any of the ROIs (i.e., DS, VS, amygdala, and dACC), nor was this moderated by group or sex (see Fig. 2B). These effects did not change upon correcting for potential confounders (i.e., ADHD, BDI, BIS, CTQ and AUDIT for the group effects; BIS for the sex effects and BIS, BDI and CTQ for the group by sex interaction effects). Cocaine-cue induced changes in self-reported negative affect or craving were not associated with cocaine cue induced activation in any of the ROIs, nor was this effect moderated by sex and/or group.

*3.2.3 Within CU group analyses between cocaine cue induced activation of the regions of interest and cocaine use characteristics*

Within the CU group, cocaine cue reactivity was unrelated to years of regular use, onset age of regular use, cocaine use per month in grams and cocaine use severity. However, the relationship between cocaine use severity (DUDIT score) and cocaine cue reactivity in the DS ($p$ = .029, $\eta^2$ = .10) and amygdala ($p$ = .024, $\eta^2$ = 0.11) was significantly moderated by sex. Follow up regression analyses with cocaine cue reactivity (i.e., reactivity to cocaine cues > neutral cues) in the amygdala and DS as dependent variables, DUDIT as predictor, and FD as covariate, demonstrated that cocaine use severity was positively associated with cocaine cue reactivity in the DS for female CUs (B = .33, $p$ = .09), but negatively in male CUs (B = -.07, $p$ = .73) (see Fig. 2C). Moreover, cocaine use severity was positively related to cocaine cue reactivity in the amygdala in female CUs (B = .31, $p$ = .12), but negatively related in male CUs (B = -.27, $p$ = .15) (see Fig. 2D).

**3.3 Emotional cue reactivity**

*3.3.1 Subjective effects*

Participants reported significantly more negative affect ($p$< .001, $\eta^2$ = .57) and lower craving scores (p<.001, $\eta^2$ =0.10), following the presentation of negative emotional cues compared to neutral cues (see Fig. 3A).. Furthermore, emotional cue induced craving was significantly moderated by group ($p$ < .01, $\eta^2$ = .10). More specifically, results demonstrated that emotional cues significantly reduced craving in CUs ($p$ < .01, $\eta^2$ = .19), but not in non-CUs. The other main and interaction effects were not significant.

*3.3.2 Group and sex differences in emotional cue reactivity and the relationship with subjective changes in negative affect*

Repeated measures analyses investigating neural activation within the ROIs, with CU group and sex as independent factors and FD as covariate of non-interest, demonstrated that emotional cues significantly increased neural activation in the DS ($p$ = .01, $\eta^2$ = .06) and amygdala ($p$ < .001, $\eta^2$ = .22) (see Fig. 3B). Moreover, emotional cue-induced activation of the amygdala was significantly stronger in females compared to males ($p$ = .046, $\eta^2$ = .04). Emotional cue induced neural activation of the ROIs did not differ between CUs and non-CUs.

*3.3.3 Within CU group analyses between emotional cue-induced activation of the regions of interest and cocaine use characteristics*

The relationship between years of regular use and emotional cue reactivity in the VS ($p$ = .013, $\eta^2$ = .13) and dACC ($p$ = < .01, $\eta^2$ = .16) was significantly moderated by sex ($p$ = .013, $\eta^2$ = .13). Follow-up regression analyses demonstrated that emotional cue reactivity in the dACC was significantly and negatively associated with years of regular use in female CUs (B = -.47, $p$ = .02), but positively in male CUs (B = .38, $p$ = .08) (see Fig. 3C). Similarly, emotional cue reactivity in the VS was significantly and negatively related to years of regular use in female CUs (B = -.54,$p$ = .002), but positively in male CUs (B = .28, $p$ = .20) (see Fig. 3D).

**3.4 Exploratory whole brain analyses**

*3.4.2 Cocaine cue reactivity*

Cocaine cues induced significant activation in the reward network, including the bilateral medial PFC, dACC, the right caudate, left amygdala, and putamen (table 3). These effects were not moderated by sex and or group.

*3.4.1 Emotional cue reactivity*

Emotional cues significantly increased activation of various regions in the salience network, including the medial prefrontal cortex, temporal cortex and visual cortex (Table 4, Fig. 4A). There was a significant group by stimulus type interaction effect in the left insula. More specifically, the left insula was more strongly activated by emotional cues in CUs compared to non-CUs (see Fig. 4B). There was also a significant stimulus type by sex interaction effect, demonstrating that the left insula and amygdala were more strongly activated in females compared to males. However, there were no significant sex-dependent differences in the neural correlates of emotional cue-reactivity between CUs and non-CUs.

### 3.5 Exploratory analyses on the influence of menstrual cycle and hormonal contraceptive use

Exploratory analyses on the influence of menstrual cycle and hormonal contraceptive use did not reveal any significant effects on cocaine cue-reactivity in any of the ROI's (supplementary material table 1). However, emotional cue induced activation of the DS was significantly stronger in females in the luteal phase of their menstrual cycle, compared to males. On the other the hand, emotional cue induced activation of the amygdala was significantly stronger in females using hormonal contraceptives compared to males (supplementary material table 2).

## 4. DISCUSSION

In the past decade, increases in cocaine use in females have been observed in comparison to males(Mustaquim *et al.* , 2021). There are indications for sex differences in clinical profiles of persons with CUD, such as psychiatric comorbidity and an accelerated progression to compulsive use in female CUs(Fonseca *et al.* , 2021). Yet, the exact neural mechanisms underlying sex differences in CUD remain poorly understood with potential implications for the development of sex-tailored treatment strategies(Orsini *et al.* , 2022). Accordingly, the main objective of the current study was to investigate sex-dependent differences in the neural activation of various regions of interest (ROIs), including the dorsal striatum (DS), ventral striatum (VS), amygdala, and dorsal anterior cingulate cortex (dACC), of both cocaine and emotional cue reactivity within CUs and non-CUs.

In contrast to our main hypotheses, no sex-dependent differences in cocaine or emotional cue reactivity were found when comparing CUs to non-CUs. Exploratory analyses demonstrated that cocaine cue-induced activation of the DS and amygdala was positively related to cocaine use severity in female CUs, whereas in male CUs only a negative relationship was observed between the cocaine-induced activation of the amygdala and cocaine use severity. Finally, emotional cue-induced activation of the dACC and VS was negatively related to years of regular use in female CUs, whereas this relationship was positive for male CUs.

The lack of sex-dependent differences in cocaine cue and emotional reactivity does not correspond with previous literature(Volkow *et al.* , 2011; Potenza *et al.* , 2012; Zhang *et al.* , 2021). A possible explanation is that the mean years of regular cocaine use in the current study sample was 4.7 and 5.2 years for male and female CUs, respectively. Previous studies consisted of a longer mean range from 11.3 and 8.4 years(Potenza *et al.* , 2012) to 18 and 20 years(Volkow *et al.* , 2011) in male and female CUs, respectively. This discrepancy might be of significance, as years of regular cocaine use has been suggested to be more centrally related to cocaine cue reactivity than CUD diagnosis(Prisciandaro *et al.* , 2015).

While the current study's main hypotheses for cocaine cue reactivity were not confirmed, exploratory analyses revealed interesting findings. First, a positive relationship was observed between cocaine cue reactivity in the DS and cocaine use severity in female CUs, whereas no relationship was observed in male CUs. This suggests that the salience of cocaine cues in female CUs become more prominent as cocaine use gets more

severe, which is commensurate with the distinctive feature of rewarding feelings of 'wanting' the drug in the later stages of addiction(Everitt & Robbins, 2013). Second, results demonstrated a positive relationship between cocaine cue reactivity in the amygdala and cocaine use severity within female CUs, whereas a negative relationship was observed in male CUs. This indicates that cocaine cues induce stronger activation in the amygdala within female CUs when severity of use is greater, whereas the opposite might be observed in male CUs. Significantly, the amygdala is a key brain region in the brain arousal/stress system that plays an important role in engaging the transition and maintenance of dependence(Koob, 2009) and reinstating cue-dependent drug seeking(Sharp, 2017), which could exacerbate the transition to compulsive use in female CUs.

Furthermore, exploratory analyses revealed significant negative relationships between emotional cue induced activation in the dACC and VS and years of regular use within female CUs. Earlier research has consistently demonstrated decreased neural response to emotional stimuli in CUD(Goldstein *et al.* , 2009; Asensio *et al.* , 2010), particularly among female CUs in the medial prefrontal cortex/ACC region(Canterberry *et al.* , 2016). This attenuated response to emotional cues is consistent with the impaired response inhibition and salience attribution model(Ceceli *et al.* , 2022) in which individuals devalue non-drug related rewards and negative stimuli that leads to risky behaviors(Canterberry *et al.* , 2016) and poor treatment outcome(Konova *et al.* , 2008). Moreover, the VS is posited to play a pivotal role in mediating responses to aversive stimuli(Konova *et al.* , 2008). Presumably this is due to the "rewarding effects" of successfully avoiding aversive or punishing events(Kim *et al.* , 2006; Oleson *et al.* , 2012), which has been shown to promote future behavior in animals(Wenzel *et al.* , 2015). Consistently, one human study demonstrated decreased activation of the NAc during passive avoidance in response to aversive stimuli, whereas greater activation of the NAc was found during active avoidance(Levita *et al.* , 2012). Taken together, this indicates that female CUs with more years of cocaine use assign less salience towards negative emotional stimuli and are less able to actively avoid aversive stimuli, primarily due to hypoactivation in the dACC and VS, respectively.

While speculative, these exploratory findings suggest that female CUs may become more amenable to positive reinforcement and compulsivity (i.e., greater DS activity), and less amenable to negative reinforcement (i.e., less dACC activity) as the addiction develops with greater severity and more years of use. Together with reinstating cue-dependent drug seeking (i.e., greater amygdala activity) and the diminished ability to actively avoid averse stimuli (i.e., less VS, but also dACC activity), this could create a double whammy for female CUs and make them more prone to risky behavior as the addiction develops, which could account for the observed "telescoping effect" in females(Fonseca*et al.* , 2021).

Finally, whole brain analyses revealed significant emotional cue-induced activation of the salience network, including the medial PFC, temporal cortex and visual cortex. Moreover, emotional-cue induced activation of the left insula was significantly stronger in CUs compared to non-CUs, but no sex-dependent differences. This is inconsistent with earlier research who demonstrated greater insula activity in female CUs compared to male CUs when exposed to stress cues(Li *et al.* , 2005; Potenza*et al.* , 2012). Potentially, this could be explained by the particular representation of emotional cues, i.e., negative valence pictures in the current study versus personalized stress imagery in earlier studies(Li *et al.* , 2005; Potenza *et al.* , 2012). Indeed, personalized cues potentially maximize cue reactivity, but simultaneously lead to heterogeneity that limits generalizability and interpretation(Ekhtiari *et al.* , 2022). Lastly, whole brain analyses demonstrated that the left insula and amygdala were more strongly activated in females compared to males in response to emotional cues, but there were no significant sex-dependent differences between CUs and non-CUs.

An important strength of the current study is its design to specifically investigate sex differences in both cocaine and emotional cue-reactivity in regular CUs and non-CUs. Specifically, the current study comprised a relatively large sample in which all individuals met DSM-5 criteria for CUD according to a self-report(First, 2015) and were matched on most cocaine-use related variables. Yet, female CUs were associated with earlier onset of regular cocaine use when compared to male CUs, but did not differ in total years of regular use or use per month (in grams). Another strength of the current study is that the negative emotional and neutral cues were obtained from the OASIS database(Kurdi *et al.* , 2017) and the cocaine cues were obtained from

an earlier study (Kaag*et al.* , 2018) that facilitates replicability for future research. Lastly, exploratory analyses were perfomed on the influence of menstrual phase and the use of hormonal contraceptives as there is increasing evidence that fluctuating sex hormones strongly influence processes of positive and negative reinforcement(Voorhees *et al.* , 2012; Kokane & Perrotti, 2020). In line with this we demonstrated that emotional cue induced activation of the amygdala and dorsal striatum was moderated by hormonal contraceptive use and menstrual phase, highlight the relevance of including measures of menstrual phase and hormonal contraceptive use in future studies.

One limitation of the current study is its cross-sectional design that precludes the ability to make any conclusions about cause and effect regarding the aforementioned relationships. Furthermore, the current study did not perform a urine screening while participants were instructed to abstain from cocaine use 24 hours prior to study participation. This is important as cocaine metabolites can be detected in urine up to six days after last use(Preston *et al.* , 2002), which is much longer than its psychopharmacological effects. Alternatively, the current study used the TLFB prior to the experiment which is a highly reliable method to assess cocaine use(Robinson*et al.* , 2014). Although several studies show a high concordance between self-report and urine screening(Darke, 1998; Wilcox *et al.* , 2013), it remains impossible to conclude that participants were not (still) intoxicated or inebriated. Finally, it may be important to conduct drug and emotional cue reactivity paradigms on separate days or randomize these conditions in order to circumvent possible carryover effects of reward and emotional cues .

To further establish the current findings, future research should take into account duration and severity of use when conducting cue reactivity paradigms by performing longitudinal and prospective research (e.g., ecological momentary assessments) in order to gain understanding in sex-dependent trajectories in positive and negative reinforcement within the development of CUD. Moreover, understanding sex differences in neural mechanisms underlying CUD is clinically significant because of differential treatment effects in both males and females(Orsini *et al.* , 2022). For instance, guanfacine is able to elicit greater reduction in stress- and cue-mediated cocaine craving in female CUs(Fox*et al.* , 2014) due to improvements in cognitive control(Milivojevic *et al.* , 2017), whereas oxytocin has shown promise in diminishing the stress response (i.e., less amygdala activation) to cocaine cues in male CUs with a history of childhood trauma, whereas it increased the stress response in female CUs with childhood trauma(Joseph *et al.* , 2020).

In conclusion, the current study found no significant sex differences in cocaine and emotional cue reactivity in regular CUs. Yet, it did demonstrate a positive relationship between cocaine cue reactivity and cocaine use severity and a significant negative relationship between emotional cue reactivity and years of regular use in female CUs compared to male CUs, thus indicating important sex differences in underlying neural mechanisms in the development of CUD. It is important to keep improving our understanding of sex differences in CUD due to its implications for treatment efficacy in both males and females.

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## AUTHORS CONTRIBUTION

AMK was responsible for the study concept, design, data acquisition, analysis. ST was responsible for preparing the first draft of the manuscript. AMK and AEG provided critical revision of the manuscript for important intellectual content. EGS was responsible for the supplement. All authors critically reviewed content and approved final version for publication.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**tables**

**Table 1**

*Demographic and clinical information in male (non-)cocaine users and female (non-)cocaine users*

| | Cocaine users | Cocaine users | C |
|---|---|---|---|
| | **Males (n = 31)** | **Females (n = 26)** | M |
| Age | 29.2 (6.8) | 26.5 (6.9) | 2 |
| Education [a] (n, %) | | | |
| Elementary school | 0 (0%) | 1 (3.7%) | 0 |
| (prevocational) secondary | 0 (0%) | 0 (0%) | 2 |
| Senior general/preuniversity | 15 (48.4%) | 13 (48.1%) | 1 |
| Higher professional/university | 16 (51.6%) | 13 (48.1%) | 1 |
| State Anxiety (STAI) | 23.8 (8.4) | 34.0 (7.5) | 3 |
| ADHD symptom severity (ADHD-RS) | 48.0 (22.7) | 50.2 (28.0) | 3 |
| Depression (BDI-II) | 9.0 (8.6) | 14.6 (8.2) | 5 |
| Impulsivity (BIS-11) | 63.8 (10.6) | 71.9 (12.6) | 5 |
| Childhood trauma (CTQ) | 48.1 (8.3) | 55.0 (15.7) | 4 |
| Framewise displacement (FD) | 0.18 (0.12) | 0.18 (0.08) | 0 |
| Menstrual phase / hormonal contraceptive use[b] | Menstrual phase / hormonal contraceptive use[b] | | |
| Follicular phase (n, %) | | 3 (11.5%) | |
| Luteal phase (n,%) | | 5 (19.2%) | |
| Hormonal contraceptive user | | 16 (61,5%) | |

Abbreviations: STAI = State-Trait Anxiety Inventory; ADHD-RS = Attention Deficit Hyperactivity Disorder-Rating Scale; BDI = Beck Depression Inventory II; BIS = Barratt Impulsiveness Scale; CTQ = Childhood Trauma Questionnaire; AUDIT = Alcohol Use Disorders Identification Test;

[a] Highest finished educational level

[b] See supplementary material for methods

**Table 2**

*Substance use characteristics in male and female cocaine users*

| | Cocaine users | Cocaine users | Main effect sex |
|---|---|---|---|
| | **Male (n = 31)** | **Female (n = 27)** | |
| Cocaine use disorder (DSM-5) [a] | | | $x^2 = 2.397$, $p = .494$ |
| No cocaine use disorder | 7 (12.1%) | 3 (5.2%) | |
| Mild cocaine use disorder | 7 (12.1%) | 4 (6.9%) | |
| Moderate cocaine use disorder | 6 (10.3%) | 7 (12.1%) | |
| Severe cocaine use disorder | 11 (19.0%) | 13 (22.4%) | |
| Age of onset regular use | 24.2 (5.3) | 21.3 (4.4) | $\mathbf{p < 0.05, \eta^2 = 0.08}$ |
| Years of use | 4.7 (4.4) | 5.2 (5.3) | $p = 0.69$, $\eta^2 < 0.01$ |
| Use per month (in grams) | 5.9 (4.9) | 3.9 (3.8) | $p = 0.10$, $\eta^2 = 0.05$ |
| Cocaine use severity (DUDIT) | 16.9 (5.9) | 16.4 (4.7) | $p = 0.74$, $\eta^2 < 0.01$ |
| Alcohol use severity (AUDIT) | 10.7 (5.8) | 12.3 (3.9) | *p = 0.15, η2 = 0.02* |

Abbreviations: DSM-5 = Fifth edition of the Diagnostic and Statistical Manual of Mental Disorders Diagnostic; DUDIT = Drug Use Disorder Identification Test for cocaine; AUDIT = Alcohol Use Disorders Identification Test.

[a] = 2–3 symptoms is mild cocaine use disorder, 4–5 symptoms is moderate cocaine use disorder, and six or more symptoms is severe cocaine use disorder.

**Table 3**

*Main and interaction effects of stimulus type (cocaine versus neutral), sex, and group*

| | Cluster pFWE cor |
|---|---|
| Cocaine cue reactivity | Cocaine cue reactivity |
| Main effect stimulus type | Main effect stimulus type |
| Cocaine cues > neutral cues | Cocaine cues > neutral cues |
| | <0,001 |
| | <0,001 |
| | <0,001 |
| | 0,007 |
| | <0,001 |
| | 0,001 |

| | Cluster pFWE cor |
|---|---|
| | <0,001 |
| | 0,009 |
| Neutral cues > Cocaine cues: no significant clusters | Neutral cues > Cocaine cues: no significant clusters |
| Stimulus type * group interaction effect: no significant clusters | Stimulus type * group interaction effect: no significa |
| Stimulus type * sex interaction effect: no significant clusters | Stimulus type * sex interaction effect: no significant |
| Stimulus type * sex * group interaction effect: no significant clusters | Stimulus type * sex * group interaction effect: no sig |

**Table 4**

*Main and interaction effects of stimulus type (emotional versus neutral), sex, and group*

| | Cluster p-value FWE-corrected |
|---|---|
| Emotional cue reactivity | Emotional cue reactivity |
| Main effect stimulus type | Main effect stimulus type |
| Emotional cues > neutral cues | Emotional cues > neutral cues |
| | <0,001 |
| | <0,001 |
| | <0,001 |
| | <0,001 |
| | 0,029 |
| Neutral cues > emotional cues | Neutral cues > emotional cues |
| | 0,035 |
| Stimulus type (emotional cues > neutral cues) * group interaction effect | Stimulus type (emotional cues > neutral cues) * |
| CU > non-CU | 0,017 |
| Non-CU > CU | *No significant clusters* |
| Stimulus type (emotional cues > neutral cues) * sex interaction effects | Stimulus type (emotional cues > neutral cues) * |
| Female > male | 0,043 |
| Stimulus type * sex * group interaction effects: *no significant clusters* | Stimulus type * sex * group interaction effects: |

## Figure legends

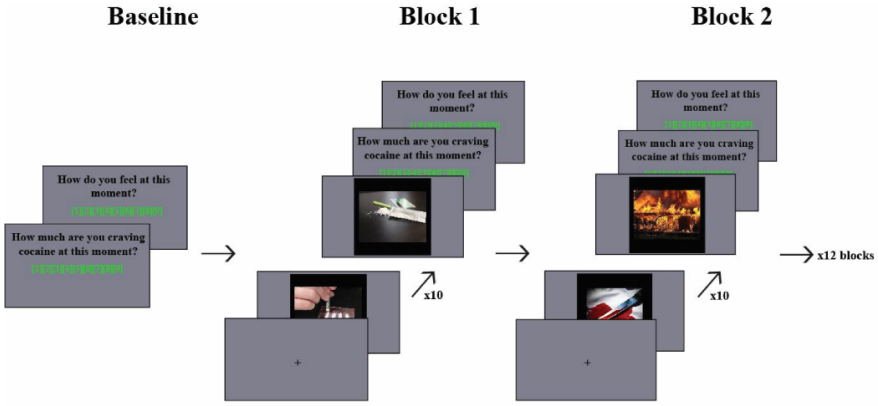



**Figure 1**

*The task started with a baseline measure of arousal ("How do you feel?") and craving ("How much are you craving cocaine at this moment?"). Then, a total of 12 blocks were shown in random order, which consisted of 3 emotionally negative blocks, 3 cocaine blocks, 3 control blocks matched to the emotionally negative blocks and 3 control blocks matched to the cocaine block. Each block consisted of 10 images (total 120 images) and were shown for 2.5 seconds. Both craving and affect were assessed after every block.*

*Figure 1*

The task started with a baseline measure of arousal ("How do you feel?") and craving ("How much are you craving cocaine at this moment?"). Then, a total of 12 blocks were shown in random order, which consisted of 3 emotionally negative blocks, 3 cocaine blocks, 3 control blocks matched to the emotionally negative blocks and 3 control blocks matched to the cocaine block. Each block consisted of 10 images (total 120 images) and were shown for 2.5 seconds. Both craving and affect were assessed after every block.

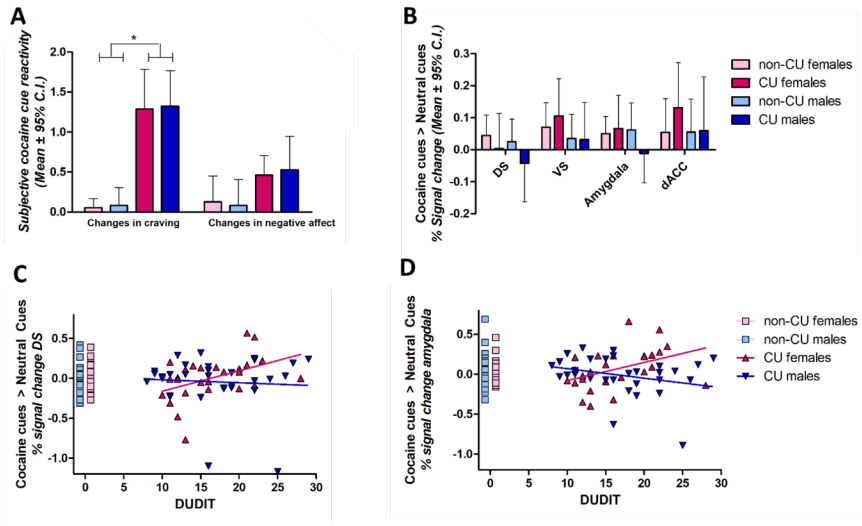



*Figure 2*

Note. A. There were no main effects of cocaine cues versus neutral cues on activation in the regions of interests. B. Self-reported craving significantly increased following cocaine cues versus neutral cues in CUs, but not in non-CUs. There were no group or sex effects on cocaine cue induced negative affect. Cocaine cue reactivity in the dorsal striatum (C) and amygdala (D) was positively associated with cocaine use severity (DUDIT scores) in female CUs, while this relation was negative in male CUs.

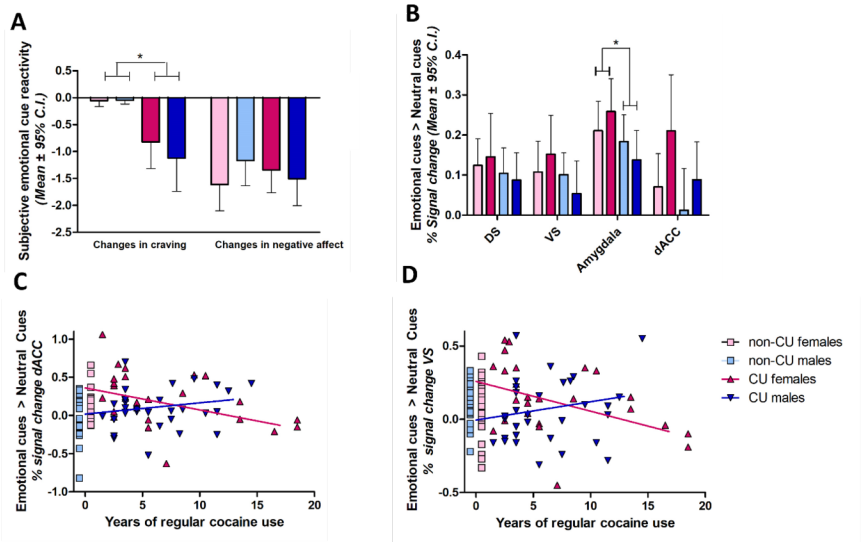



*Figure 3*

Note. A. There was a significant effect of emotional versus neutral cues in the dorsal striatum and amygdala. B. emotional cues significantly reduced craving in CUs, but not in non-CUs. Emotional cues significantly reduced affect in both groups. Emotional cue reactivity in the dACC (C) and ventral striatum (D) was significantly and negatively associated with years of regular cocaine use in CU females, while this relationship was positive in male CUs.

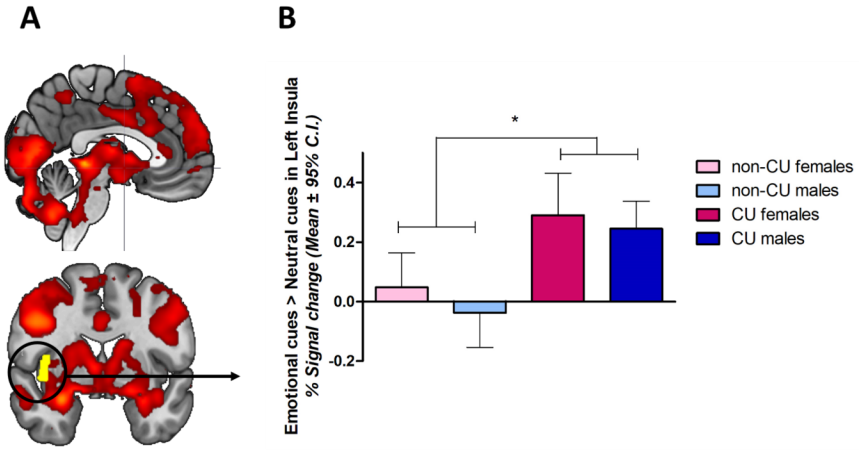
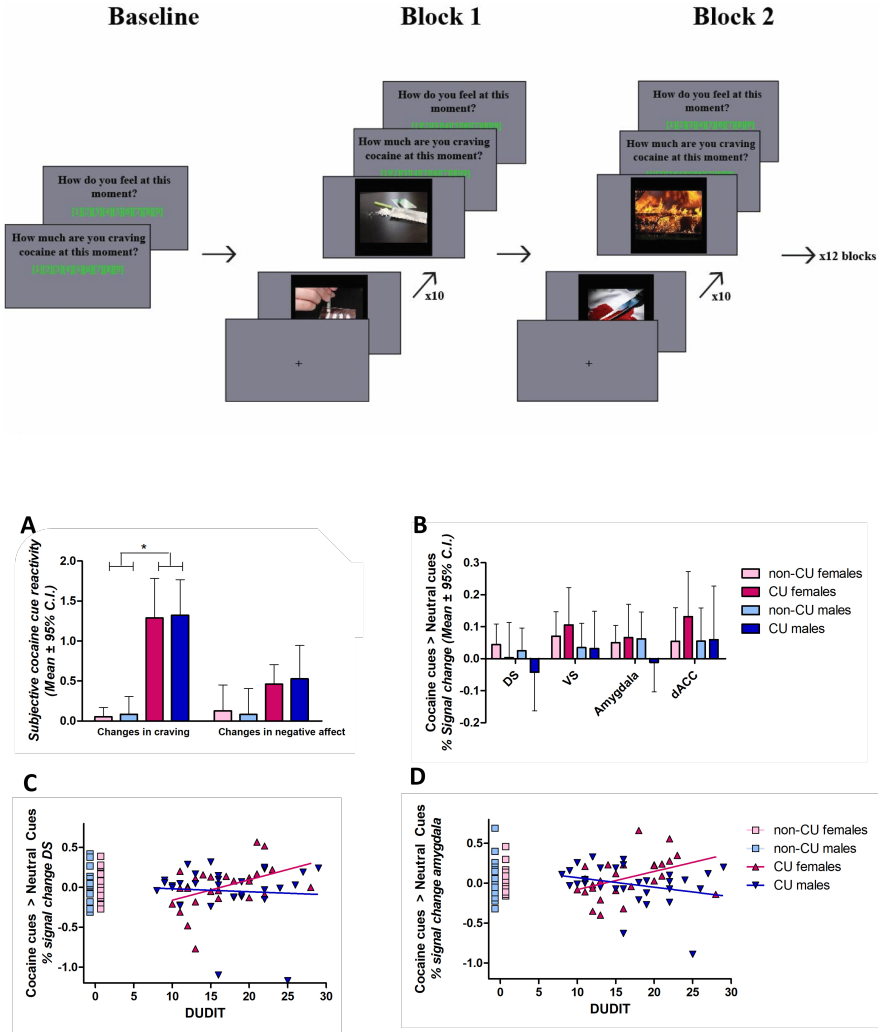
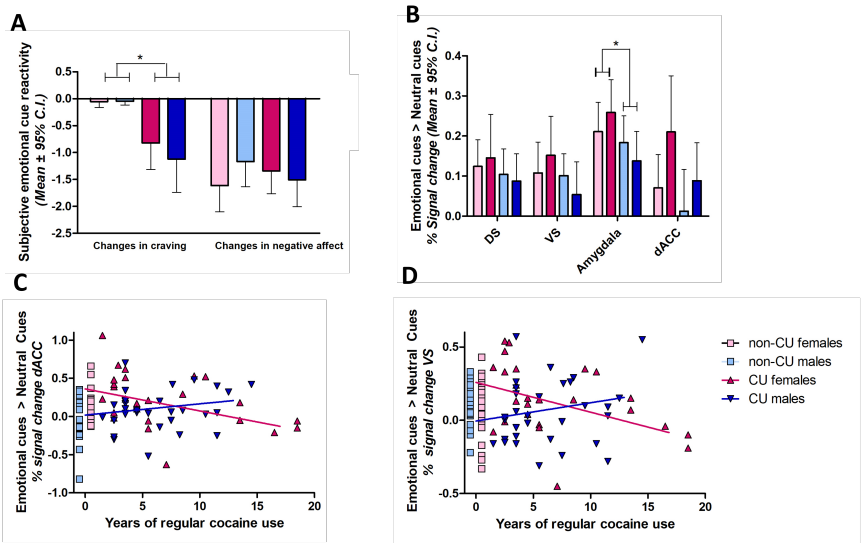


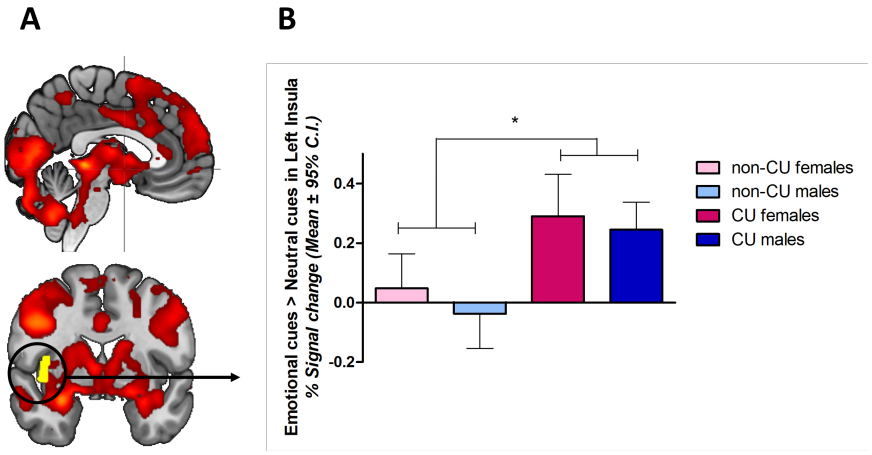

*Figure 4*

Note. A. Emotional cues significantly increased activation of the salience network. B. There was a significant

group by stimulus type interaction effect in the left insula.



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Tables.docx available at https://authorea.com/users/627199/articles/648266-sex-dependent-differences-in-the-neural-correlates-of-cocaine-and-emotional-cue-reactivity-in-regular-cocaine-users-and-non-drug-using-controls-understanding-the-role-of-duration-and-severity-of-use