Are cocaine users too sensitive? Functional and structural brain imaging studies in regular cocaine users
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Chapter 4

Hyper-responsiveness of the neural fear network during fear conditioning and extinction learning in male cocaine users

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Abstract

Objective: To investigate whether cocaine use disorder is associated with abnormalities in the neural underpinnings of aversive conditioning and extinction learning as these processes may play an important role in the development and persistence of drug abuse.

Method: In this study 40 male regular cocaine users and 51 male controls, underwent a fear conditioning and extinction protocol during functional magnetic resonance imaging scanning. Skin-conductance response was measured throughout the experiment as an index of conditioned responses.

Results: Cocaine users showed hyper-responsiveness of the amygdala and insula during fear conditioning and also hypo-responsiveness of the dorsomedial prefrontal cortex during extinction learning. In cocaine users, but not in controls, skin-conductance responses were positively correlated to responsiveness of the insula, amygdala and dorsomedial prefrontal cortex during fear conditioning, but correlated negatively to responsiveness of the ventromedial prefrontal cortex during extinction learning.

Conclusions: Increased sensitivity to aversive conditioned cues in cocaine users might be a risk factor for stress-relief craving in cocaine use disorder. These results support the postulated role of altered aversive conditioning in cocaine use disorder and may be an important step in understanding the role of aversive learning in the pathology of cocaine use disorder.
**Introduction**

Cocaine is the second most used illicit drug in Europe, particularly among young adult males and is often used in combination with alcohol and cannabis. There are no registered pharmacological treatments for cocaine addiction, although cognitive behaviour therapy and contingency management have shown to be fairly successful. Learning processes, including Pavlovian and instrumental conditioning, play an essential role in the development and persistence of substance use disorder. While these learning processes may form a potential treatment target, psychotherapies targeting conditioning have not (yet) shown to be effective. A possible reason is that previous research primarily focused on the learning mechanisms underlying appetitive conditioning (cue-exposure treatment), while aversive conditioning may be equally important in the etiology and treatment of cocaine use disorder.

Through the process of appetitive conditioning, drug-responses become associated with drug-related cues. When these drug-related cues are subsequently encountered in an abstinent state, they can trigger the retrieval of memories of prior drug experiences and thereby induce cue-reactivity, craving, drug-seeking and drug-taking behavior. However, there is increasing evidence that aversive conditioning plays an equally important role in substance use disorder: (i) Stress-induced relief craving is a frequently observed phenomenon in addiction and addicted individuals are thought to take drugs to avoid aversive states such as stress. Through the process of aversive conditioning, external stimuli can become associated with internal stress states, thereby (indirectly) motivating drug-seeking and drug-taking behavior. (ii) There is a high comorbidity between anxiety-disorders and substance use disorder. Since abnormalities in aversive conditioning and extinction learning have been reported in anxiety disorders, similar abnormalities are expected to be found in substance use disorder. (iii) The neural underpinnings of appetitive and aversive conditioning are thought to largely overlap since the mesolimbic dopamine system is a key player in both types of conditioning and extinction learning. As a consequence, neuroadaptive changes within the mesolimbic dopamine system due to stress or drug-use may also modulate the processing of appetitive conditioning or aversive conditioning, respectively. Knowledge about the role of aversive conditioning in substance use disorder could thus have important implications for understanding its pathophysiology and the development of prevention and treatment strategies. However, so far no studies are available on the neural and physiological underpinnings of aversive conditioning in substance use disorder.

From studies in healthy individuals we know that aversively conditioned stimuli evoke an increase in skin-conductance response and activation of the neural fear network including the amygdala, dorsomedial prefrontal cortex and insula and deactivation of the ventromedial prefrontal cortex. Within this network the amygdala, dorsomedial prefrontal cortex and insula are involved in the expression of aversive conditioned responses, while the ventromedial prefrontal cortex is involved in the inhibition of conditioned behaviour. Anxiety disorders are characterised by hyper-responsiveness of the amygdala, dorsomedial prefrontal cortex and insula during fear learning and hypo-responsiveness of the ventromedial prefrontal cortex during extinction learning, reflecting the presence of enhanced fear learning and impaired fear extinction capabilities. Counterintuitively, these differences in neural plasticity are typically not associated with enhanced differential skin-conductance responses.

In this study we investigated the physiological (skin-conductance responses) and neural correlates (regional brain activation) of fear conditioning and extinction in cocaine abusers and controls. While the neural and physiological underpinnings of aversive conditioning and extinction learning have not yet been investigated in substance use disorder, previous studies demonstrated that substance use disorder is associated with hyper-responsiveness of the dorsomedial prefrontal cortex, insula and amygdala to conditioned drug cues, a response which is slowly extinguished. We therefore hypothesized that cocaine abuse is associated with enhanced fear conditioning and impaired extinction learning as reflected by hyper-activation of the amygdala, insula and dorsomedial prefrontal cortex during fear conditioning and hypo-activation of the ventromedial prefrontal cortex during extinction learning as compared to controls.
Methods and materials

Participants
Seventy male regular cocaine users and 73 male controls were included in this study. Complete skin-conductance response and MRI datasets were collected from 58 controls and 53 cocaine users. An additional 4 controls and 3 cocaine users were excluded because of MRI artefacts, resulting in the inclusion of 54 controls and 48 cocaine users. All participants were males (aged 18-50) recruited through local advertisement in the greater Amsterdam area in the Netherlands. Cocaine users were actively using cocaine and currently non-treatment seeking. Cocaine users were included when using cocaine at least once per week for a minimum period of 6 months. All participants were screened using the MINI International Neuropsychiatric Interview. Exclusion criteria for all participants were: major medical or neurological disease, lifetime history of psychotic or bipolar disorder, the use of antidepressants/antipsychotics or a contraindication for MRI scanning. Controls were excluded if they met DSM-IV criteria for life-time substance abuse or dependence, and in case of any psychotropic medication use, other than antidepressants/antipsychotics. Alcohol, cocaine and cannabis use in the 6 months before study inclusion was quantified using the timeline-follow back procedure. Smoking severity was measured using the Fagerstrom Test for Nicotine Dependence, state anxiety was measured with the state-trait anxiety inventory (STAI) and premorbid verbal intelligence was estimated using the Dutch version of the National Adult Reading Test. The study was approved by the Ethical Review Board of the Academic Medical Centre of the University of Amsterdam, the Netherlands. All subjects gave written informed consent.

Experimental paradigm
Briefly, the classical fear conditioning paradigm, which was conducted in the MRI scanner, consisted of a habituation, conditioning and extinction phase. The conditioned stimuli (CS) were yellow and blue squares, of which one (the CS+) was followed by the unconditioned stimulus (US) in 33% of the conditioning trials. The CS- was never followed by the US. The US was an aversive electrical shock to the wrist. The shock intensity was set individually to be highly annoying but not painful. Skin-conductance responses were measured simultaneously with fMRI acquisition. Additional details are provided in the supplement.

Functional magnetic resonance imaging data acquisition and analysis
Images were acquired on a 3.0-T Philips Achieva scanner and analyzed using SPM8 including standard preprocessing and first-level modelling (see supplement). In accordance to previous studies, first level contrasts were computed for the CS+(unpaired to the US) and the CS- during the early and late conditioning phase and early and late extinction phase in order to assess temporal gradation of signal intensity. Early and late phases included the first and the last half of the CS+ and CS- trials, respectively. These contrast images were entered into a second level full-factorial design to investigate task and group effects.

A whole-brain correlation analysis was performed to investigate whether individual differences in conditioned responses measured by skin-conductance and neural responses (fMRI) were associated, and whether these correlations during fear conditioning and extinction learning differed between groups. An additional correlation whole-brain analysis was performed to test for the possible confounding effects of state anxiety and the amount, type and days since last substance use (see supplementary data).

All analyses were family-wise error rate corrected for multiple comparisons (cluster p<0.05, height threshold p<0.01). A small-volume correction was applied for the amygdala (p<0.05) because of its a priori role in aversive conditioning. With regard to the correlation analyses, interactions that assessed group differences in correlations were tested and followed by within group correlation analyses when significant.
**Statistical analysis**

Group differences in clinical characteristics were assessed using independent-samples T-tests or non-parametric tests when appropriate. Stimulus induced skin-conductance responses were defined as difference between maximum and minimum responses within 8 seconds after CS onset. Responses divided by the largest response for that individual to account for individual differences and square root transformed (+1) to normalize the data. Subjects were said to show conditioned responses when differential skin-conductance responses were positive in the first or second half of the conditioning phase. A repeated measures analysis of variance was used, including CS-type (CS+: CS−), phase (conditioning, extinction) and time (early, late) as within-subjects factors, and group (controls, cocaine users) as between-subjects factor.

**Results**

**Clinical characteristics**

To enable the assessment of extinction learning, successful acquisition of fear conditioning is required. Of all subjects, 51 controls (94%) and 40 cocaine users (83%) showed a positive differential skin-conductance response during early or late conditioning. These percentages did not differ significantly ($\chi^2 = 3.26, p=0.109$).

In line with previous studies, the non-conditioners were excluded based on these physiological data and all further analyses were therefore conducted on these subsamples (controls: n=51, cocaine users: n=40). Groups were of similar age and IQ, but cocaine users scored significantly higher on state anxiety and weekly alcohol intake, and had more comorbid DSM-IV diagnoses of depression and anxiety disorder (Table 1). On average, cocaine users used 7.6 grams on 8.7 separate days on a monthly basis with an onset age of 19.4 years for an average period of 8.8 years of cocaine use and 3 days since last use. All cocaine users met the DSM-IV criteria of cocaine dependence or abuse. In addition, 86% of the cocaine users also smoked nicotine and 45% also used cannabis on a (more than) weekly basis.

**Table 1. Clinical and demographic characteristics of non-drug using controls and regular cocaine users**

<table>
<thead>
<tr>
<th>Clinical and demographic variable</th>
<th>controls n=51</th>
<th>SD or IQR</th>
<th>Cocaine users n=40</th>
<th>SD or IQR</th>
<th>p-value</th>
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</thead>
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<tr>
<td>Age</td>
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<td>6.43</td>
<td>31.0</td>
<td>7.9</td>
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<td>IQ</td>
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<td>9.2</td>
<td>101.4</td>
<td>8.4</td>
<td>n.s.</td>
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<tr>
<td>Shock intensity</td>
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<td>1.8</td>
<td>101.4</td>
<td>8.4</td>
<td>n.s.</td>
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<td>State anxiety (STAI)</td>
<td>28</td>
<td>7.0</td>
<td>35</td>
<td>17.0</td>
<td>&lt;0.001</td>
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<tr>
<td>Alcohol use (units per week)</td>
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<td>3.5</td>
<td>19.9</td>
<td>35.4</td>
<td>&lt;0.001</td>
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<td>Smoking severity (FTND)</td>
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<td>-</td>
<td>5</td>
<td>2.0</td>
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<tr>
<td>Grams of cocaine use per month</td>
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<td>-</td>
<td>7.6</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Years of cocaine use</td>
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<td>-</td>
<td>8.8</td>
<td>6.4</td>
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</tr>
<tr>
<td>Frequency (days/month)</td>
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<td>-</td>
<td>8.7</td>
<td>5.8</td>
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<tr>
<td>Onset age of cocaine use</td>
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<td>-</td>
<td>19.4</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Days since last use</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td>2.0</td>
<td></td>
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<td>Prevalence of smoking, depression and anxiety disorders</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>% Smoking</td>
<td>0%</td>
<td>86.0%</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Lifetime history of depression</td>
<td>5.9%</td>
<td>32.5%</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Lifetime history of anxiety disorder</td>
<td>0%</td>
<td>10%</td>
<td>0.032</td>
<td></td>
<td></td>
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</table>

* Values represent mean ± standard deviation (SD)

b Values represent median ± interquartile range (IQR)

c According to the DSM-IV
Physiology
The shock intensity between groups did not differ significantly (controls: median=24mA, cocaine users: median=28 mA, U=701, z=-1.26, p>0.05). The skin-conductance response during conditioning and extinction revealed significant CS-type by phase interaction ($F_{3,87}=10.78$, $p<0.001$). As expected, due to excluding participants that did not show successful fear conditioning, paired-sample t-tests revealed that both groups showed a significant CS+/CS- differences on skin-conductance responses during early conditioning ($t_{90}=4.62$, $p<0.001$ and late conditioning ($t_{90}=7.39$, $p<0.001$), but also during early extinction ($t_{90}=3.24$, $p=0.002$). No significant CS+>CS- differences in skin-conductance response were displayed during late extinction ($t_{90}=1.53$, $p=0.129$), indicating successful extinction learning. Similar to previous findings in anxiety disorders, there was no significant group by CS-type by phase interaction, indicating equal levels of fear conditioning and extinction learning between groups on a physiological level (Figure 1).

![Figure 1. Mean skin-conductance response in cocaine users and controls. There was a significant differential skin-conductance responses during early and late conditioning and early extinction, in both groups. No significant group differences in skin-conductance response were demonstrated.](image)

Neuroimaging
The 4-way interaction between group (cocaine users, controls), phase (conditioning, extinction), time (early, late) and CS-type (CS+; CS-) was non-significant. Because fear conditioning and extinction are qualitatively distinct processes and neural responses during fear learning show a strong temporal gradation in signal intention, group differences in fear learning were investigated for each phase separately, in line with previous studies.

Fear conditioning
During fear conditioning, both groups displayed significant activation in the neural fear network for the CS+>CS- contrast, including the insula, dorsomedial prefrontal cortex, amygdala, and the superior temporal cortex (supplementary table S1), reflecting fear conditioning on the neural level. Between group analysis revealed that, compared to controls, cocaine users showed recruitment of the left amygdala and several cerebellar and occipital regions during early conditioning (Figure 2A, table 2) and enhanced recruitment of the left insula and rolandic operculum during late conditioning (Figure 2B, table 2). These results are consistent with the hypothesis that cocaine users display neural hyper-responsivity during fear conditioning. In addition, conditioned skin-conductance responses and conditioned neural responses were significantly correlated across subjects (Supplementary table S2). An interaction analysis showed that the correlation between the skin-conductance response and activity of the right amygdala and several regions within the prefrontal cortex was significantly different between groups (Figure 2C, Figure 2D, table 2). Follow-up tests showed a significant positive correlation in cocaine users but not in controls.
Extinction learning

During early and late extinction, the CS+→CS- contrast was associated with significant activation of the insula, dorsomedial prefrontal cortex and supramarginal gyrus. The CS-→CS+ contrast was associated with significant activation of the ventromedial prefrontal cortex, the superior and middle frontal cortex and several regions within the occipital and temporal cortex (Supplementary table S1). There were no significant between group differences during early extinction. However, during late extinction, cocaine users showed reduced activation in the dorsomedial prefrontal cortex compared to controls (Figure 3A, table 2). This suggests that cocaine users exhibit enhanced extinction of dorsomedial prefrontal cortex fear responses.

Similar to the findings obtained during conditioning, an interaction analysis showed that the correlation between the skin-conductance response and activity of the ventromedial prefrontal cortex and parietal cortex was significantly different between groups during early extinction (Figure 3B, table 2). Within group analyses showed a significant negative correlation in cocaine users but not in controls. In addition, the correlation between skin-conductance response and activation in the insula, left amygdala and bilateral superior temporal gyrus was significantly different between groups during late extinction (Figure 3C, table 2). Within group analysis showed a significant positive correlation in cocaine users, but not in controls. These results suggest that reduced fear expression on a physiological level is related to enhanced ventromedial prefrontal cortex activity and reduced dorsomedial prefrontal cortex activity.

The effect of state anxiety and poly-substance use

To explore the effects of the level of substance use, light and heavy users of nicotine, cocaine, alcohol and cannabis were compared. The neural correlates of aversive conditioning were unrelated to state anxiety, the amount and type of substance (cocaine, cannabis, alcohol and nicotine) used or days since use. During extinction learning, there was a negative relation between the amount of cannabis used and responsiveness of the superior temporal, middle temporal and inferior frontal cortex and a positive relation between the amount of cocaine used and responsiveness of the left insula, lateral prefrontal cortex, the parietal and occipital cortex (supplementary figure S1A/S1B, table S3). Nicotine and alcohol use were unrelated to the neural correlates of extinction learning. Regression analysis showed that state anxiety was negatively correlated with activation of the cerebellum during late extinction (supplementary figure S1C,table S3).
Figure 3. The neural correlates of extinction learning and the correlation with skin-conductance responses. Cocaine users displayed hypoactivation of the dorsomedial prefrontal cortex during late extinction (A). Cocaine users displayed significant stronger negative correlations between activation of the ventromedial prefrontal cortex (MNI: 10 44 -8) and skin-conductance responses during early extinction (B) and significant stronger positive correlations between activation of the neural fear network, including the left insula (MNI: -38 -28 -12) and skin-conductance responses during late extinction (C). Scatterplots represent the beta weight of the peak-voxel resulting from the whole brain analysis against the skin-conductance responses during early (D) and late extinction (E).
<table>
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<th>Early Conditioning (CS+&gt;CS-)</th>
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<th>Cluster P-value</th>
<th>Voxel z-value</th>
<th>Peak voxel MNI-coordinates</th>
<th>Voxel region</th>
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<td></td>
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<td>4.84</td>
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All results were p<0.05, cluster level family-wise error corrected with an initial height threshold of p=0.01 uncorrected

*Corrected for the volume of the right amygdala, p_{peak voxel}<0.05
+ Significant positive correlation
- Significant negative correlation
□ Non-significant relation
Discussion

Although aversive conditioning and extinction learning have been suggested to play an important role in the development and persistence of drug abuse\textsuperscript{140,147,156}, this is the first study addressing the physiological and neural mechanisms underlying aversive conditioning and extinction learning in substance use disorder. Consistent with the hypothesis of enhanced aversive conditioning in substance use disorder, cocaine users showed hyper-activity of the amygdala and insula during aversive conditioning compared to controls. Inconsistent with the hypothesis of impaired extinction learning, cocaine users showed hypo-activation of the dorsomedial prefrontal cortex during late extinction, suggesting enhanced extinction learning compared to controls.

While there were no group differences in skin-conductance response during aversive conditioning or extinction learning, skin-conductance response and activation of the neural fear network were more strongly correlated across subjects in cocaine users than in controls, suggesting that the emotional response to conditioned stimuli is stronger in cocaine users than in controls. Overall these findings support the postulated role of abnormal aversive learning processes in substance use disorder.

The amygdala is important for the rapid encoding of new stimulus-threat relationships\textsuperscript{154}, while the insula modulates (the visceral response to) conditioned\textsuperscript{156}. The dorsomedial prefrontal cortex is suggested to be involved in conscious negative appraisal of threat. These brain regions are key structures within a neural fear network and previous studies have repeatedly demonstrated that activation of these regions is: (i) associated with fear conditioning and extinction learning\textsuperscript{154}, (ii) enhanced in individuals with enhanced fear learning and impaired extinction learning\textsuperscript{157} and (iii) related to other measures of conditioned behaviour, including skin-conductance response\textsuperscript{152}. The finding of increased amygdala and insula activation during fear conditioning and reduced dorsomedial prefrontal cortex activation during late extinction in cocaine users therefore suggests that cocaine users display enhanced fear conditioning as well as enhanced extinction learning compared to controls.

Increased amygdala activity in cocaine users during early conditioning may reflect increased attention for threat-related stimuli and enhanced aversive conditioning, followed by increased activity of the insula during late conditioning which may reflect enhanced visceral processing. Increased responsiveness of these structures within the neural fear network are thought to underlie negative reinforcement mechanisms in substance use disorder, stress induced relief craving and subsequent continuation of or relapse into drug use\textsuperscript{1,21,156,161}. Abnormalities in the neural underpinnings of aversive conditioning in cocaine users may therefore reflect a risk for cocaine abuse and relapse.

In contrast to our hypotheses, cocaine users showed reduced activity of the dorsomedial prefrontal cortex during late extinction, suggesting the presence of superior, and not impaired, extinction learning. While enhanced fear extinction learning may be beneficial in anxiety disorders, it may actually form a risk for the persistence of drug use, as it may underlie an inability to modify behaviour in response to negative outcomes, resulting in risky behaviour\textsuperscript{162}. Future studies should however investigate whether these difference are also present after reinstatement of the fear conditioned response. Irrespectively of whether enhanced extinction of neural fear conditioned responses is good or bad, our data demonstrates that the neural underpinnings of fear extinction learning in cocaine users differ from those in patients with an anxiety disorder\textsuperscript{149,153,154,159,163}. While the relation between enhanced fear extinction learning and stress relief-craving remain to be investigated, these findings may explain why cue-exposure treatment, which is a successful treatment strategy in anxiety disorders, is not as effective in addiction\textsuperscript{141}.

In line with the typical observation in anxiety disorders, we found no group differences in differential skin-conductance responses\textsuperscript{149,158,159}. Although these results could be interpreted as less efficient neural processing in cocaine users, there is substantial evidence that hyper-activation of the amygdala, insula and dorsomedial prefrontal cortex reflects the persistence of an increased expression of conditioned fear\textsuperscript{154}. However, there
are several other explanations for the dissociation between the skin-conductance response and fMRI results. First, differential skin-conductance responses are suggested to be more dependent on higher cognitive levels of learning, whereas differential activation of the neural fear network is suggested to be independent of higher cognitive processing\(^{152}\). This would suggest that cocaine abuse is associated with abnormalities in fear conditioning and fear extinction that are mainly dependent on unconscious processes. Furthermore, the finding that the skin-conductance response and fMRI data are significantly correlated in cocaine users but not in controls may indicate that skin-conductance responses in cocaine users are less dependent on conscious processing of conditioned cues. Alternatively, it has demonstrated that the amygdala plays a critical role in the modulation of skin-conductance responses to threat\(^{164}\). Therefore, these results may indicate that cocaine users have a stronger emotional response to stimuli that predict an aversive outcome. In addition, several studies in anxiety disorders have demonstrated that, while there were no group differences in skin-conductance responses during fear conditioning or extinction learning, differences were presented during extinction recall\(^{159}\). Thus it could be that differences in neural processing precede differences in skin-conductance responses, that can be detected only during extinction recall. Altogether, skin-conductance responses may not be sensitive and fear-specific enough to detect small group differences during fear conditioning or extinction learning\(^{165}\).

While the sensitivity for stress has long been known to be an important risk factor for substance use and relapse\(^{146}\), this is one of the first studies to investigate the potential neural mechanism that underlie this phenomenon. We demonstrated that the neural fear network of regular cocaine users is hyperresponsive to cues that predict a negative outcome. These findings emphasize that in addition to reducing drug-conditioned responses (reward craving), treatment should also try to reduce the (neural) sensitivity to stressors (relief craving). This could be achieved by means of cognitive behavioural treatment (e.g. mindfulness-based relapse prevention\(^{166}\) or pharmaceutical treatments that target the noradrenergic stress-system (e.g. propranolol\(^{167}\)).

An important strength of this study is the large sample size and the assessment of the potential confounding effect of state anxiety. However, the study also has limitations. First, because only male participants were included in the study these results cannot simply be generalized to female cocaine users. Second, because of the cross-sectional design, we need to be very cautious with statements about the causality of our findings and future studies should examine whether increased neural sensitivity for aversive events is a risk factor for cocaine abuse, a consequence of cocaine abuse, or a combination of both. Third, most cocaine users in the current sample also used cannabis, alcohol and nicotine on a regular basis, thereby making it impossible to tell whether differences in aversive conditioning are related to cocaine, cannabis or alcohol use or to some combination of these. Nevertheless, the exploratory analysis suggested that hyper-responsiveness of the amygdala and insula during aversive conditioning and hypo-responsiveness of the dorsomedial prefrontal cortex during late extinction learning are independent of the type or amount of substance used. Moreover, as poly-drug use is common among cocaine users in treatment\(^{4}\), we expect the investigated sample to reflect the typical cocaine users. Fourth, we tested for group differences during early and late conditioning, as well as early and late extinction, while there was no phase by group interaction effect. Although this is in accordance with most studies in the field\(^{154}\), it should be noted that such statistical flexibility could increase type I errors. Finally, while the neural pathways that underlie aversive and appetitive-conditioning and extinction are overlapping, more research is needed to investigate whether and how enhanced neural sensitivity to aversive conditioned cues and enhanced sensitivity to drug-conditioned cues are related.

In summary, this is the first study to show that cocaine use disorder is associated with hyper-responsiveness of the neural fear network during fear conditioning and extinction learning, possibly reflecting enhanced fear learning. Although the relation between aversive conditioning and stress-induced relief craving remains to be investigated, this study is an important contribution to the understanding of the role of aversive conditioning in, and the etiology of, substance use disorder.
Supplementary material

Fear conditioning and extinction measurements and task

In this study a classical fear conditioning paradigm was used. Briefly, this paradigm consisted of a habituation, conditioning and extinction phase. The conditioned stimuli (CS) consisted of yellow and blue squares and the unconditioned stimulus (US) was an aversive electrical stimulation to the participant’s wrist. Before the imaging session, MR compatible carbon electrodes (Kendall H135TSG) for electrical stimulation were placed on the right wrist 1-2 cm apart. All subjects selected a level of shock intensity that was experienced as highly annoying but not painful, to be used in the experiment. Intensities could vary between 1 and 99 mA with a constant voltages of 400 V.

In the habituation phase, the 4 CS+ and 4 CS- were presented in a pseudo-random manner. During the conditioning phase, the CS+ was paired with the US at a partial reinforcement rate of 33%. There were 18 CS- trials, 12 CS+ trials that were unpaired with the US and 6 CS+ trials that were paired with the US. The US directly followed the offset of the CS+. After a break of approximate 30 seconds the extinction phase began. During this phase 18 CS+ trials and 18 CS- trials were presented, but none of the CS+ trials were paired with an electrical shock. For each trial during the experiment, the CS+ and CS- were presented for 4 seconds, the US was presented for 2 ms, and the intertrial interval varied between 6.5 and 9.5 seconds during which a fixation cross was presented. Before onset of each phase subjects were instructed that they could receive electrical shocks and that they should pay attention on the relation between the visual stimuli presented and the electrical shocks.

FMRI data acquisition and first-level analysis

Images were acquired on a 3.0-T Achieva full-body scanner (Philips Medical Systems, Best, the Netherlands) using a 32 channel SENSE head coil. Echo planar images (EPIs) were taken covering the whole brain, with a total of 37 ascending axial slices (3x3x3mm voxel size; slice gap 3mm; TR/TE 2000ms/28ms; matrix 80x80). Also a T1-3D high resolution anatomical scan (TR/TE 8.2/3.7; matrix 240x187; 1x1x1 voxel; transverse slices) was taken.

fMRI data were analyzed using SPM8. Preprocessing included realignment, slice-time correction, coregistration of the structural and functional scans, normalization to MNI-space based on the segmented structural scan and smoothing with a kernel of 8 mm full-width at half maximum. First level models included separate regressors for CS-, CS+ paired with the US, CS+ unpaired with the US and the US itself, during habituation, conditioning and extinction blocks. These regressors were convolved with the canonical hemodynamic response function. Six realignment parameters were included as regressors of no interest. A high pass filter (1/128 Hz) was included in the first level model to correct for low frequency signal drift.

Physiological data acquisition and analysis

Skin conductance was measured simultaneously with fMRI acquisition. Skin-conductance response (SCRs) was measured using an MRI compatible GSR set with Ag/AgCl electrodes covered in isotonic gel (Brain Products GmbH, Germany) with a constant voltage of 0.5V. Electrodes were placed at the medial phalanges of the index and middle finger. SCR was recorded using Net Station (version 4.5.2) at a sample rate of 250 Hz. SCR was recorded from the onset to the end of the fear conditioning paradigm. The MRI artefact was removed using the MRI artefact removal tool in NetStation. Subsequently, the SCR signal was low-pass filtered using a cut-off value of 2 Hz.
Exploring the effects of poly-substance abuse on the neural correlates of aversive conditioning and extinction learning

The relation between state anxiety and days since last use was tested by adding these covariates to the fMRI model, for early conditioning, late conditioning, early extinction and late extinction. To explore whether abnormalities in aversive conditioning and extinction were related to the amount of substance used, we aimed to test the relation between cannabis, nicotine, alcohol and cocaine use and the neural correlates of aversive conditioning and extinction learning. The data of drug use could not be transformed to have a normal distribution. Therefore cocaine users were divided into mild or severe users of alcohol, nicotine and cocaine, based on the median split mild alcohol use < 19.9 glasses a week, severe alcohol use ≥ 19.9 glasses a week; no or mild nicotine use < FTND score of 5, severe nicotine use ≥ FTND score of 5; mild cocaine use < 7.6 grams per month, severe cocaine use ≥ 7.6 grams per month. Cannabis use was based on whether or not they used less than weekly (no to mild use ranging from 0 to 3.8 days per month) or more than weekly (severe user ranging from 4.3 to 30.3 days per month). Chi-square tests indicated that there was no relation between mild or severe use of the four substances.
Supplementary table S1. Neural correlates of aversive conditioning and extinction: main task effects

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**CS-> CS+**

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**Late extinction**

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All results were p<0.05, cluster level family-wise error rate corrected with an initial height threshold of p=0.01 uncorrected.

*Corrected for the volume of the right amygdala, p_peak corrected<0.05
All results were p<0.05, cluster level family-wise error corrected and p<0.01 voxel level uncorrected

### Late extinction (CS+ > CS-)

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### Early extinction (CS+ > CS-)

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### Early Conditioning (CS+ > CS-)

#### Positive correlations

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### Late extinction (CS+ > CS-)

#### Positive correlation

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*Corrected for the volume of the right amygdala, p<0.05 voxel level family-wise error corrected*
Supplementary table S3. Neural correlates of aversive conditioning and extinction: exploring the relation with state anxiety, and the type and amount of substance used

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All results were p<0.05, cluster level family-wise error corrected and p<0.01 voxel level uncorrected
Figure S1. The association between cannabis use and state anxiety on the neural correlates of fear conditioning and extinction learning

S1A: Early extinction: Heavy < Mild cannabis use

S1B: Late extinction: Heavy < Mild cannabis use

S1C: Late extinction: Heavy > mild cocaine use

S1D: Late extinction: negative correlation state anxiety

*Figure S1. The association between cannabis use and state anxiety on the neural correlates of fear conditioning and extinction learning*