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# Normalizing effect of heroin maintenance treatment on stress-induced brain connectivity

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Recent evidence has shown that a single maintenance dose of heroin attenuates psychophysiological stress responses in heroin-dependent patients, probably reflecting the effectiveness of heroin-assisted therapies for the treatment of severe heroin addiction. However, the underlying neural circuitry of these effects has not yet been investigated. Using a cross-over, double-blind, vehicle-controlled design, 22 heroin-dependent and heroin-maintained outpatients from the Centre of Substance Use Disorders at the University Hospital of Psychiatry in Basel were studied after heroin and placebo administration, while 17 healthy controls from the general population were included for placebo administration only. Functional magnetic resonance imaging was used to detect brain responses to fearful faces and dynamic causal modelling was applied to compute fear-induced modulation of connectivity within the emotional face network. Stress responses were assessed by hormone releases and subjective ratings. Relative to placebo, heroin acutely reduced the fear-induced modulation of connectivity from the left fusiform gyrus to the left amygdala and from the right amygdala to the right orbitofrontal cortex in dependent patients. Both of these amygdala-related connectivity strengths were significantly increased in patients after placebo treatment (acute withdrawal) compared to healthy controls, whose connectivity estimates did not differ from those of patients after heroin injection. Moreover, we found positive correlations between the left fusiform gyrus to amygdala connectivity and different stress responses, as well as between the right amygdala to orbitofrontal cortex connectivity and levels of craving. Our findings indicate that the increased amygdala-related connectivity during fearful face processing after the placebo treatment in heroin-dependent patients transiently normalizes after acute heroin maintenance treatment. Furthermore, this study suggests that the assessment of amygdala-related connectivity during fear processing may provide a prognostic tool to assess stress levels in heroin-dependent patients and to quantify the efficacy of maintenance treatments in drug addiction.

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**Abbreviations:** DCM = dynamic causal modelling; OFC = orbitofrontal cortex

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# Introduction

Dysfunction of stress pathways increases relapse vulnerability in drug-addicted individuals (Sinha, 2008). Stress-induced drug craving and hypothalamus-pituitary-adrenal axis responses are even predictive of the outcome of cocaine relapse (Sinha *et al.*, 2006), thus suggesting potential biological markers for relapse propensity. The aversive state in drug addiction is partly mediated by activation of corticotropin-releasing factor in prefrontal and limbic brain areas, such as the orbitofrontal cortex (OFC) and the amygdala (Koob and Kreek, 2007; Koob, 2013). The OFC has direct projections to the amygdala (Ghashghaei *et al.*, 2007) and is functionally connected with the amygdala during the integration of emotional and motivational information (Schoenbaum and Roesch, 2005). For example, human neuroimaging studies reveal that the OFC and the amygdala are coactivated during withdrawal-induced craving (Koob and Volkow, 2010), probably through downstream modulation of the OFC on limbic regions (Rolls, 2004). OFC–amygdala interactions may thus reflect connections of vulnerability that are susceptible to relapses after periods of abstinence.

A study in heavy alcohol drinkers showed that acute alcohol ingestion reduced functional connectivity between the right amygdala and OFC during fearful face processing (Gorka *et al.*, 2013), an effect that was only evident for the right amygdala–OFC connectivity, whereas the left coupling seemed to be sensitive only for positive emotions. Given that acute alcohol intoxication reduces subjective and physiological responses to stress (Sayette *et al.*, 1992; Hefner and Curtin, 2012), the authors concluded that this reduced right amygdala-OFC interplay may lead to reduced negative affect after alcohol intoxication (Gorka *et al.*, 2013). In the same vein, depressed patients with bipolar disorder showed significantly greater right prefrontal–amygdala connectivity during fearful face processing than healthy controls, which was reduced after antidepressant treatment (Perlman *et al.*, 2012). These findings are in line with studies showing the impact of stress on the right OFC (Hanson *et al.*, 2010) and on right OFC–amygdala connectivity (Fan *et al.*, 2014).

The prescription of pharmaceutical heroin has repeatedly been found to be an effective treatment for severe heroin addiction (van den Brink *et al.*, 2003; Haasen *et al.*, 2007; Oviedo-Joekes *et al.*, 2009) and for heroin addicts who have failed to benefit from methadone maintenance treatment (Blanken *et al.*, 2010), although methadone maintenance failures may often be due to low dosing levels (Strain *et al.*, 1993; Farré *et al.*, 2002). Compared with healthy control subjects, chronic heroin users exhibit increased functional connectivity between the right amygdala and the OFC during resting state (Ma *et al.*, 2010). Although recent evidence has demonstrated that a daily maintenance dose of heroin acutely reduced levels of stress hormones, withdrawal, craving and anxiety (Walter *et al.*, 2013), it has not yet been explored whether this heroin-induced attenuation of psychophysiological stress responses is also mirrored in reduced OFC-amygdala connectivity during fearful processing, analogously to the effect of acute alcohol ingestion.

The processing of facial affects provides a suitable framework for the study of OFC-amygdala interactions. That is, fearful face processing underlies integrated activity between the inferior occipital gyrus that receive input from the retina, the fusiform gyrus, which already processes facial valences, the amygdala and the ventral prefrontal cortex, subsuming the OFC, which evaluates emotional stimuli (Haxby *et al.*, 2002; Fusar-Poli *et al.*, 2009*b*). Within this visual-limbic-prefrontal network, the amygdala is thought to play a key role in the rapid detection of facial affect and in orchestrating network integrity during evaluation of affective stimuli (Adolphs, 2008; Pessoa and Adolphs, 2010). It has been suggested that prefrontal regions, such as the OFC, regulate amygdala activity during evaluation of emotional face stimuli (Nomura *et al.*, 2004). However, visual processing of fearful face emotions is also mediated through a re-entry mechanism of connections from the visual cortex to amygdala and connections from the amygdala to the visual cortex (Vuilleumier *et al.*, 2004; Herrington *et al.*, 2011). This visual-limbic coupling during fearful face processing can be increased by psychosocial stress (Li *et al.*, 2014) and hyper-connectivity between the amygdala and visual cortex may underlie increased negative emotion processing and anxiety (Frick *et al.*, 2013). We have previously reported significant heroin effects on amygdala activity during fear processing in heroin-dependent patients compared to a placebo treatment (acute heroin withdrawal) (Schmidt *et al.*, 2014), which suggests abnormalities in the underlying connectivity, in particular to the visual cortex (i.e. fusiform gyrus).

In this study, the objective was to detect how heroin acutely modulated right OFC–amygdala connectivity compared to a placebo treatment in heroin-dependent patients (experimentally-induced state of acute withdrawal; Schmidt *et al.*, 2014) and healthy controls. In particular, we used functional MRI and dynamic causal modelling (DCM; Friston *et al.*, 2003) to assess modulation of OFC–amygdala connectivity during fearful face processing. DCM explicitly allowed us to compute the directionality of fear-induced connectivity among the face network (Dima *et al.*, 2011; Diwadkar *et al.*, 2012; Volman *et al.*, 2013) and how this is altered after pharmacological manipulations (Grefkes *et al.*, 2010; Schmidt *et al.*, 2013). Besides exploring acute heroin effects on amygdala–OFC connectivity, the second aim of this study was to address whether the acute heroin effect on local amygdala activity (Schmidt *et al.*, 2014) was due to abnormal connectivity from the fusiform gyrus. Finally, to validate the biological basis of our model, we further tested whether the amygdala-related connectivity strengths were related to previously reported psychophysiological stress responses in these patients (Walter *et al.*, 2013). We hypothesized that heroin injection would

reduce amygdala-related connectivity within the fear network relative to the placebo treatment, in accompaniment to its effect on psychophysiological stress responses (Walter *et al.*, 2013). Notably, fearful faces were not used to stress participants, but rather to assess whether amygdala-related connectivity strengths during fearful face processing differentiated between the stress-related placebo treatment and the heroin treatment.

# Materials and methods

The presented DCM analyses are based on the functional MRI data previously published by Schmidt *et al.* (2014). Details of image acquisition and the mass-univariate statistical parametric mapping analyses are described in the previous publication and in the Supplementary material. Here, we provide a summary of the participants' characteristics, study design and details of data analysis with DCM and Bayesian model selection. Furthermore, data on psychophysiological stress responses and plasma levels were taken from recently published studies (Walter *et al.*, 2013, 2014). As these data had to be adapted explicitly to subjects who completed the face paradigm, a brief description of these methods is also provided.

## Clinical trial registration information

Clinical trial information can be found at http://clinicaltrials.gov/show/NCT01174927. Name: Effects of Diacetylmorphine (DAM) on Brain Function and Stress Response. Number: NCT01174927.

## Participants

The study population consists of 17 healthy control subjects [females/males: 4/13; mean age ± standard deviation (SD): 42.2 ± 2.6 years] and 22 patients with opioid dependence according to ICD-10 criteria (females/males: 8/14; mean age ± SD: 41.1 ± 7.2 years). All participants were smokers and groups were matched for age [$F(1,37) = 0.173$; $P = 0.680$], gender ($\chi^2 = 0.742$; $P = 0.389$) and cannabis consumption (four healthy control subjects and seven patients, $\chi^2 = 0.325$; $P = 0.567$). Patients had a past history of intravenous heroin consumption, with current maintenance for at least 6 months, and with an unchanged heroin dose during the previous 3 months. In more detail, the patient age at first heroin use was 19.09 ± 3.27 years, with a current duration of dependence of 21 ± 6.40 years and a daily maintenance dose of 309.55 ± 121.48 mg (methadone-equivalent doses of 77.39 ± 30.37 mg). Patients were excluded from participation if they currently had additional physical diseases or a psychiatric disorder including other comorbid conditions such as substance dependencies. We did not exclude patients with a past history of psychiatric disorders or substance dependence. Clinically experienced psychiatrists (M.W.) conducted a structured clinical interview for DSM-IV Axis II Disorders to assess the diagnosis of comorbid personality disorders. Patients were told to abstain from illicit drug consumption during the study and to abstain from alcohol intake and smoking 72 and 2 h before scanning, respectively. Thus, patients with a positive alcohol breathalyzer test were excluded. Nevertheless, urine samples of nine patients (no healthy controls) were tested positive for cocaine at one or both points of the measurement ($\chi^2 = 9.041$; $P = 0.003$). We have incorporated this potential confounder into our analyses. Healthy control subjects were recruited from the general population by advertisement in the same geographical area. Participants who consumed >20 g alcohol per day, or who had any psychiatric, neurological, or severe medical illness history were excluded. After complete description of the study to the subjects, written informed consent was obtained.

## Study design

Placebo and heroin were administered through an indwelling intravenous catheter over a period of 30 s, using a cross-over, double-blind, vehicle-controlled design. Heroin hydrochloride was dissolved on site in 5 ml sterile water and aspirated into a syringe, as previously described (Stohler *et al.*, 1999). Each patient was scanned twice, with a short interval between scans (mean 9 ± 3.8 days). Of the 22 patients included, 11 received heroin at the first scan and saline at the second. Those subjects who received heroin before scanning were administered vehicle after scanning, whereas the subjects who received saline before scanning were administered heroin after scanning. Patients received two doses of heroin per day, one before/after scanning in the morning, and one in the evening. Thus, the last regular heroin injection was 12 h before the experiment. The healthy controls participated only in the placebo condition.

## Fear processing paradigm

Study subjects participated in one 6-min experiment with event-related functional MRI (Fusar-Poli *et al.*, 2009*a*), in which they were presented with 10 different facial identities, each expressing 50% or 100% intensities of fear or a neutral expression.(Young *et al.*, 2002). There were thus 30 different facial stimuli in total; each face was presented twice for 2 s. The order of facial identities and expression type was pseudo-randomized, such that there was no successive presentation of the same facial expression type. The interstimulus interval was varied from 3 to 8 s according to a Poisson distribution, with an average interval of 5.9 s; the individuals then viewed a fixation cross. The face paradigm was conducted ~30 min after treatments.

## Assessment of psychophysiological stress responses and plasma levels

Craving ('desire to use heroin') was assessed 60 min after placebo/heroin treatment using the 45-item Heroin Craving Questionnaire (Tiffany, 1999), which measures positive and negative aspects of craving on five theory-derived nine-item scales. The German version of the State-Trait Anxiety Inventory was used to quantify state-anxiety after both treatments in patients as well as in healthy controls (Spielberger *et al.*, 1970). Symptoms of withdrawal were reported on a visual analogue scale (values ranging from 0 to 10). Samples of adrenocorticotropic hormone and cortisol were collected through an intravenous catheter at baseline, 20 and 60 min after substance administration. Salivary cortisol was analysed

with a time-resolved immunoassay with fluorescence detection. Total cortisol concentrations were measured in serum with the Immulite 2000 Cortisol-Test (Siemens). The measurement range of the test is at 1–50 µg/dl; the analytical sensitivity is 0.20 µg/dl. Adrenocorticotropic hormone (ACTH) was measured in EDTA plasma with the ACTH Immulite-Test (Siemens). The intra-assay precision was <6.1% for concentrations >50 pg/ml; the interassay precision was 9.4% for concentrations >51 pg/ml. The plasma concentrations of heroin (diacetylmorphine) and morphine were measured in venous ammonium-heparinized plasma and assessed using high-performance liquid chromatography on a 125 × 2 mm i.d. Nucleosil 50 C-8 ec column with a particle size of 5 µm and a 8 × 3 mm i.d. precolumn packed with Nucleosil 120 C-8 and a particle size of 3 µm followed by diode-array detection. Sample preparation and instrumental conditions were as described previously (Bourquin *et al.*, 1999).

## Network connectivity analysis

### Dynamic causal modelling

DCM (Friston *et al.*, 2003) is a generic Bayesian system identification technique used to compute effective connectivity between brain regions for inferences related to 'hidden' neurophysiological mechanisms. In DCM for functional MRI, the dynamics of the neural states underlying regional blood oxygen level-dependant response are modelled by a bilinear differential equation that describes how the neural states (x) change over time (t) as a function of endogenous interregional connections (matrix A), as well as modulatory effects on these connections (matrix B), and driving inputs (matrix C) (Friston *et al.*, 2003; Stephan *et al.*, 2007). The endogenous connections represent coupling strengths in the absence of input to the system, whereas the modulatory effects represent task-specific alterations in this connectivity. In this study, we focused on the fear-induced modulation of visual-limbic-prefrontal connections (modulatory effect), but also examined heroin effects on endogenous connections and driving inputs.

$$f(x,u) = \frac{dx}{xt} = \left(A + \sum_{i=1}^{m} uB^{(j)}\right)x + Cu$$

### Volumes of interest

We selected visual-limbic-prefrontal volumes of interest in accordance with previous DCM studies of emotional face processing (Fairhall and Ishai, 2007; Dima *et al.*, 2011; Herrington *et al.*, 2011; Goulden *et al.*, 2012; Sladky *et al.*, 2013; Volman *et al.*, 2013). In particular, these volumes of interest comprised the bilateral fusiform gyrus and amygdala and the right OFC. The bilateral fusiform gyrus and amygdala were selected based on studies indicating bilateral activity in these regions during fearful versus neutral face processing (Fusar-Poli *et al.*, 2009*b*). As the aim of this study was to explore whether the acute heroin-induced reduction of stress hormone release, craving and anxiety, was reflected in altered brain connectivity during fearful face processing, we only incorporated the right OFC as prefrontal region into our model, on the basis of following sources: (i) evidence showing that the right OFC can be critically influenced by stress (Hanson *et al.*, 2010), as well as its connectivity to the right amygdala (Fan *et al.*, 2014); (ii) a previous study in heavy alcohol drinkers, which showed that acute alcohol ingestion reduced functional connectivity between the right amygdala and right OFC during fearful face processing (Gorka *et al.*, 2013), thus suggesting that this reduced right amygdala–OFC connectivity may lead to reduced negative affect after alcohol intoxication (Sayette *et al.*, 1992; Hefner and Curtin, 2012). In this study, we tested whether acute heroin treatment in dependent patients induced a similar effect; and (iii) previous studies emphasizing the association of the right OFC during states of drug craving (Volkow *et al.*, 1999). The coordinates were based on the maxima across all subjects for the contrast of fearful minus neutral faces within the same anatomical area as defined by the PickAtlas toolbox (Fig. 1A) (Maldjian *et al.*, 2003). For each subject, time series from these regions of interest were extracted within spheres of 10-mm radii centred on the peak for the effects of interest F contrast ($P < 0.05$, adjusted) as previously performed (Sladky *et al.*, 2013).

### Defining model architecture

Starting from this initial model, we created three different variations per model, in which fearful faces (modulatory effect) were allowed to modulate the endogenous connections (Fig. 1B). All presented stimuli (neutral and fearful faces) were entered into the bilateral fusiform gyrus, the visual input region of our model. These three variations, namely modulation of (i) forward connection; (ii) backward connection; or (iii) both were guided by studies emphasizing the modulation of bottom-up or top-down connectivity induced by facial affect among the fusiform gyrus, amygdala and OFC (Nomura *et al.*, 2004; Vuilleumier *et al.*, 2004; Fairhall and Ishai, 2007; Dima *et al.*, 2011; Herrington *et al.*, 2011). Although previous studies indicated interhemispheric functional connectivity between the fusiform gyrus and the amygdala (Irwin *et al.*, 2004; Davies-Thompson and Andrews, 2012), this interhemispheric visual and limbic coupling has been found during resting state and during the presentation of faces versus objects or places. In other words, while the model captured inter-hemispheric visual and limbic connectivity induced by the driving input of all stimuli, fearful faces were not allowed to modulate these interhemispheric endogenous connections. A graphical overview of our model architecture is depicted in Fig. 1.

### Bayesian model selection and averaging

Bayesian model selection is an essential procedure of DCM studies, as it can be used to test competing hypotheses for the neural mechanisms generating the data. Bayesian model selection rests on comparing the evidence of a predefined set of models (the model architecture). A random-effects Bayesian model selection approach has been suggested for group studies, as this is capable of quantifying the degree of heterogeneity in a population, while being extremely robust to potential outliers (Stephan *et al.*, 2009). The probability that one model is more likely than any other model can be expressed by the exceedance probability of each model. After inferring the most likely network architecture underlying a specific neural process, one can compare the parameter estimates obtained from Bayesian model selection for between-group inferences.

Statistical comparison of model parameter estimates across groups is only valid if those estimates stem from the

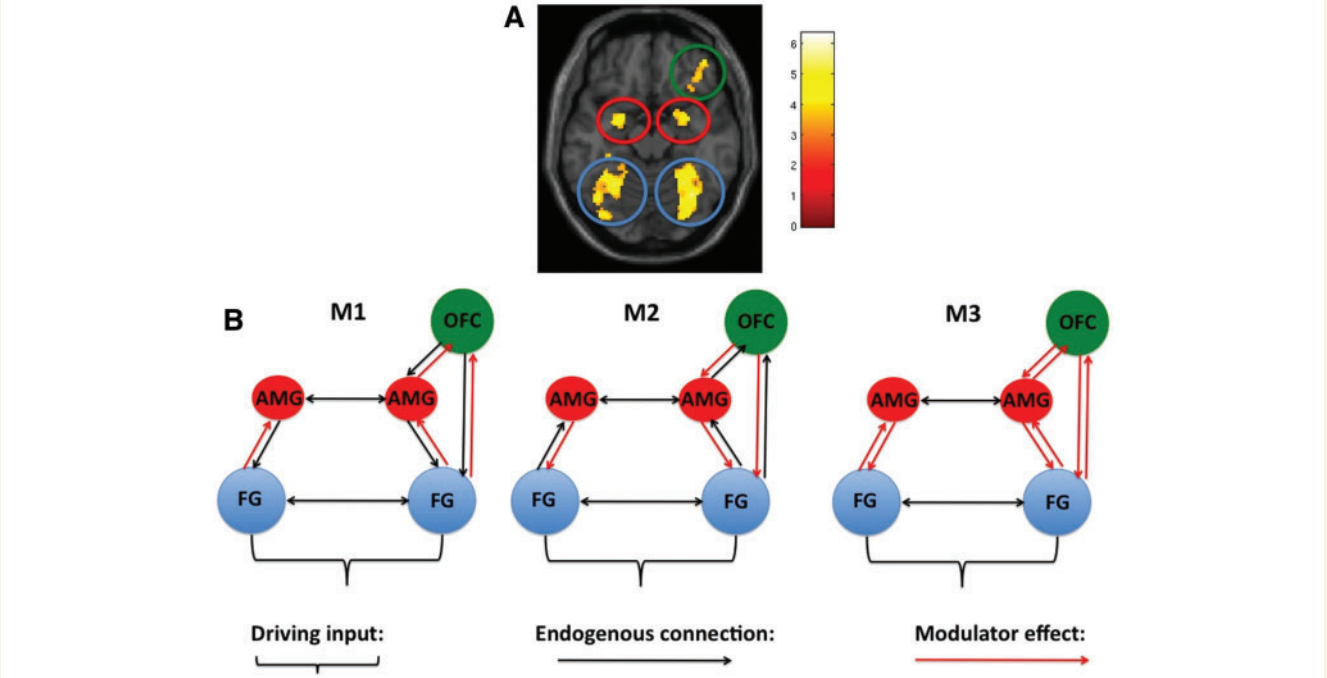


**Figure 1 Model architecture.** (**A**) Task-related brain activation within the anatomical mask during fearful versus neutral face processing in the right ($x = 32$, $y = -70$, $z = -10$; cluster size 895) and left fusiform gyrus ($x = -38$, $y = -62$, $z = -22$; cluster size 1081), right ($x = 26$, $y = -4$, $z = -14$; cluster size 271) and left amygdala ($x = -20$, $y = -2$, $z = -22$; cluster size 229) and right OFC ($x = 34$, $y = 24$, $z = -8$; cluster size 736). Activations are corrected for FWE cluster-level at $P < 0.05$ with a voxel-level threshold of $P < 0.001$. (**B**) Specification of model architecture. The sources comprising the models were the bilateral fusiform gyrus (FG), bilateral amygdala (AMG) and right OFC. From this basic layout, three models were created by considering where the modulatory effect of fearful faces might be expressed, namely on forward (M1), backward (M2) or bilateral connections (M3) among the visual-limbic-prefrontal network.

same model. If different models or families are found to be optimal across groups, Bayesian model averaging has been recommended as the standard approach for clinical DCM studies (Seghier *et al.*, 2010; Stephan *et al.*, 2010). Bayesian model averaging averages posterior parameter estimates over models, weighted by the posterior model probabilities (Penny *et al.*, 2010). Thus, models with a low posterior probability contribute little to the estimation of the marginal posterior.

### Group statistics of dynamic causal modelling parameters

After Bayesian model averaging, we used the resulting posterior means from the averaged DCM for examining differences among groups. Statistical analysis of group differences in connection strengths concerned the posterior means of coupling estimates, when using Bayesian model averaging for all three models. In other words, we compared the parameter estimates from participant-specific DCMs that were averaged over the three models (by using Bayesian model averaging) separately within each group and treatment. Thus, we were able to test for differences in 12 parameters describing the endogenous connections (matrix A), study how eight of these parameters were modulated by fearful faces (modulatory effect, matrix B), and use two parameters to describe the driving input bilateral into the fusiform gyrus. Paired *t*-tests were used to compare the heroin and placebo treatment in patients, whereas two sample *t*-tests were used to compare patients with healthy control subjects. The statistical threshold was adjusted for multiple comparisons using the Bonferroni correction. Thus, the threshold was adjusted by dividing the *P*-value by the number of connections tested (12, eight and two, respectively).

# Results

## Network connectivity analysis

### Optimal model search

Model 2, incorporating the modulation of backward connections, was clearly the best fitting model in healthy controls (exceedance probability: 70%). Although Model 2 was also superior to other models after the heroin treatment (exceedance probability: 36%), it was only marginally superior to Model 1 (exceedance probability: 34%) and Model 3 (exceedance probability: 30%). In the placebo condition, there was a preference for Model 1 (modulation of forward connections) (exceedance probability: 40%), followed by Model 3 (exceedance probability: 30%) and Model 2 (exceedance probability: 29%). Bayesian model selection results are depicted in Fig. 2.

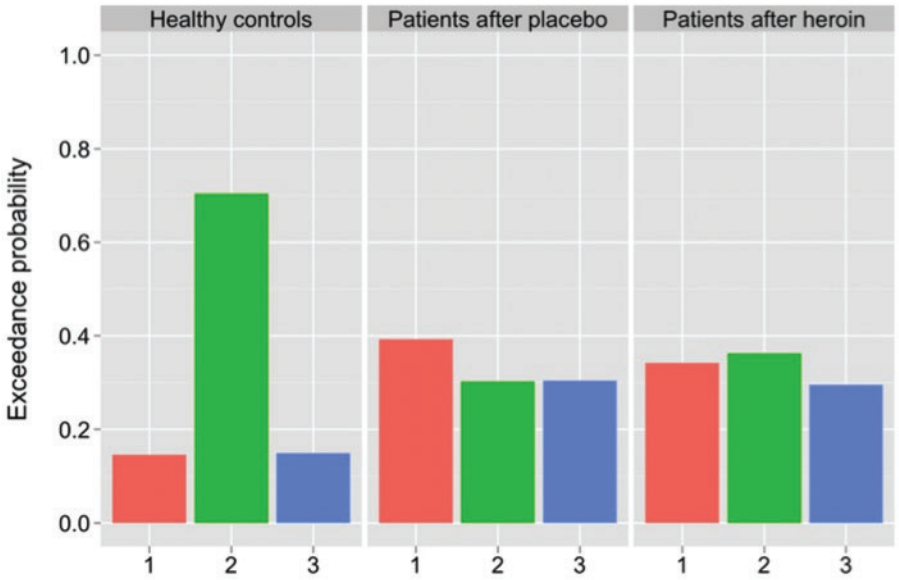


**Figure 2 Bayesian model selection results.** Bayesian model selection results among all three dynamic causal models. The models differed in whether they included fear-induced modulation of forward (Model 1), backward (Model 2) or both forward and backward connections (Model 3) among the visual-limbic-prefrontal network. Results for each treatment are expressed in terms of exceedance probability, the relative probability that this model is more likely than any other of the models tested, given the treatment data.

## Heroin effects on driving input and endogenous connections

We found no heroin effect on driving inputs relative to the placebo treatment in patients, neither for the input into the left [$t(21) = 0.207$, $P = 0.838$] nor for the input into the right fusiform gyrus [$t(21) = -0.608$, $P = 0.550$]. Relative to healthy controls, no significant effects on the driving inputs were found after heroin [left fusiform gyrus: $F(1,37) = 0.744$, $P = 0.872$; right fusiform gyrus: $F(1,37) = 0.003$, $P = 0.255$] and placebo treatment in patients [left fusiform gyrus: $F(1,37) = 0.534$, $P = 0.973$; right fusiform gyrus: $F(1,37) = 0.815$, $P = 0.558$].

No significant differences were found between the heroin and placebo treatment in patients regarding endogenous connections, or between the heroin treatment in patients and healthy controls. Compared with healthy controls, patients after placebo treatment manifested increased connectivity from the left fusiform gyrus to the left amygdala [$F(1,37) = 6.183$, $P = 0.029$, uncorrected for multiple comparisons]. Posterior parameters after Bayesian model averaging for driving inputs and endogenous connections are depicted in Table 1.

## Heroin effect on the fear-induced modulation of connectivity

Relative to placebo in patients, heroin significantly reduced the fear-induced modulation of connectivity from the left fusiform gyrus to the left amygdala [$t(21) = 2.95$, $P = 0.006$, corrected for multiple tests] and from the right amygdala to the right OFC [$t(21) = 2.38$, $P = 0.027$, uncorrected] (Fig. 3). Importantly, this heroin effect was found to be independent of whether patients consumed cocaine ($n = 9$) [fusiform gyrus→amygdala: $F(1,8) = 4.46$, $P = 0.002$, corrected; amygdala→OFC: $F(1,8) = 2.44$, $P = 0.041$, uncorrected] or not ($n = 13$) [fusiform gyrus→amygdala: $F(1,12) = 3.02$, $P = 0.011$, uncorrected; amygdala→OFC: $F(1,12) = 2.19$, $P = 0.049$, uncorrected].

Compared with healthy controls, patients under placebo exhibited a significantly higher fear-induced modulation of connectivity from left fusiform gyrus to the amygdala [$F(1,37) = 19.16$, $P = 0.002$, corrected] and from the right amygdala to the right OFC ([$F(1,37) = 3.89$, $P = 0.045$, uncorrected] (Fig. 3). It is noteworthy that these increased connectivity estimates were evident in patients without [fusiform gyrus→amygdala: $F(1,28) = 14,69$, $P = 0.001$, corrected; amygdala→OFC: $F(1,28) = 3.18$, $P = 0.046$, uncorrected] or with cocaine consumption [fusiform gyrus→amygdala: $F(1,24) = 10,03$, $P = 0.001$, corrected; amygdala→OFC: $F(1,24) = 0.73$, $P = 0.048$]. No difference was found between healthy controls and patients under heroin, regardless of whether cocaine was consumed or not. Bayesian model averaging results for each treatment separately are summarized in Table 2.

## Plasma levels

Heroin (diacetylmorphine) plasma concentrations decreased from 898 ng/ml (SD: 807) to 170 (SD: 260) and 8 (SD: 33) ng/ml at 3, 10 and 60 min, respectively. 6-Acetylmorphine exhibited a similar time-concentration profile to that of

**Table 1 DCM driving inputs and endogenous connections**

| | Healthy controls | Placebo treatment | Heroin treatment |
|---|---|---|---|
| **Driving inputs** | | | |
| Left FG | −0.0187 (0.0224)* | −0.0185 (0.0243)* | −0.0206 (0.0338)* |
| Right FG | −0.0264 (0.0272)* | −0.0120 (0.0248)* | −0.016732 (0.0240)* |
| **Endogenous connections** | | | |
| Left FG→right FG | −0.0068 (0.0830) | 0.0474 (0.0929)* | 0.0353 (0.1206) |
| Left FG→left AMG | 0.1500 (0.0626)* | 0.0626 (0.1452) | 0.1535 (0.1071)* |
| Right FG→left FG | 0.0053 (0.1322) | 0.0643 (0.1233)* | 0.0564 (0.0996) |
| Right FG→right AMG | 0.0084 (0.1876) | 0.0278 (0.1405) | 0.1105 (0.1242)* |
| Right FG→OFC | 0.0679 (0.1572) | 0.0065 (0.1728) | 0.0943 (0.1008)* |
| Left AMG→left FG | −0.0024 (0.0345) | −0.0034 (0.0416) | 0.0028 (0.0478) |
| Left AMG→Right AMY | 0.0061 (0.0316) | 0.0123 (0.0198)* | 0.0209 (0.0308)* |
| Right AMG→Right FG | −0.0005 (0.0395) | 0.0074 (0.0422) | −0.0017 (0.0378) |
| Right AMG→left AMG | 0.0141 (0.0212)* | 0.0134 (0.0310) | 0.0313 (0.0332)* |
| Right AMG→OFC | 0.0120 (0.0120)* | 0.0174 (0.0364)* | 0.0170 (0.0280)* |
| OFC→right FG | −0.0135 (0.0235)* | 0.0071 (0.0397) | 0.0005 (0.0269) |
| OFC→right AMG | 0.0137 (0.0420) | 0.0214 (0.0370)* | 0.0155 (0.0237)* |

Values are mean (SD). Values were obtained after Bayesian model averaging across all three dynamic causal models. FG = fusiform gyrus; AMG = amygdala. *Significant *t*-tests within each group compared with zero (*P*'s < 0.05).

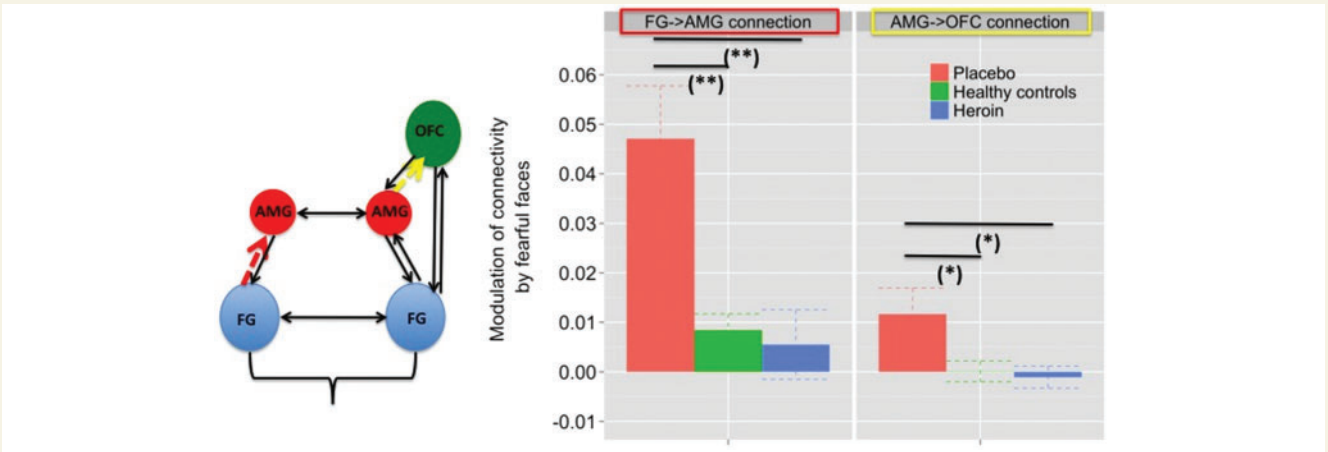


**Figure 3 Differences in fear-induced modulation of effective connectivity among groups and treatments.** Fear-induced modulation of the left fusiform gyrus→amygdala and right amygdala→OFC connectivity for each treatment condition. Significant differences between healthy controls and patients after placebo and heroin administration are indicated at *$P < 0.05$ uncorrected and at **$P < 0.00625$ corrected for multiple comparisons. Error bars indicate standard errors. FG = fusiform gyrus; AMG = amygdala.

**Table 2 DCM parameter estimates for the modulation of connectivity induced by fearful faces**

| Modulatory parameters | Healthy controls | Patients under placebo | Patients under heroin |
|---|---|---|---|
| Left FG→Left AMG | 0.0084 (0.0129)* | 0.0471 (0.0502)* | 0.0055 (0.0330) |
| Right FG→Right AMG | −0.0063 (0.0229) | 0.0055 (0.0595) | 0.0145 (0.0359) |
| Right FG→Right OFC | −0.0099 (0.0332) | 0.0245 (0.0813) | −0.0063 (0.0512) |
| Left AMG→Left FG | −0.0052 (0.0246) | −0.0013 (0.0114) | 0.0020 (0.0119) |
| Right AMG→Right FG | −0.0002 (0.0119) | 0.0009 (0.0120) | −0.0017 (0.0095) |
| Right AMG→Right OFC | 0.0001 (0.0081) | 0.0072 (0.0114)* | −0.0011 (0.0104) |
| Right OFC→Right FG | −0.0031 (0.0066) | 0.0014 (0.0117) | −0.0006 (0.0059) |
| Right OFC→Right AMG | 0.0037 (0.0104) | 0.0009 (0.0095) | 0.0015 (0.0097) |

Values for the modulatory effect of fearful faces were obtained after Bayesian model averaging across all three dynamic causal models. FG = fusiform gyrus; AMG = amygdala; *Significant *t*-tests within each group compared with zero (*P*'s < 0.05).

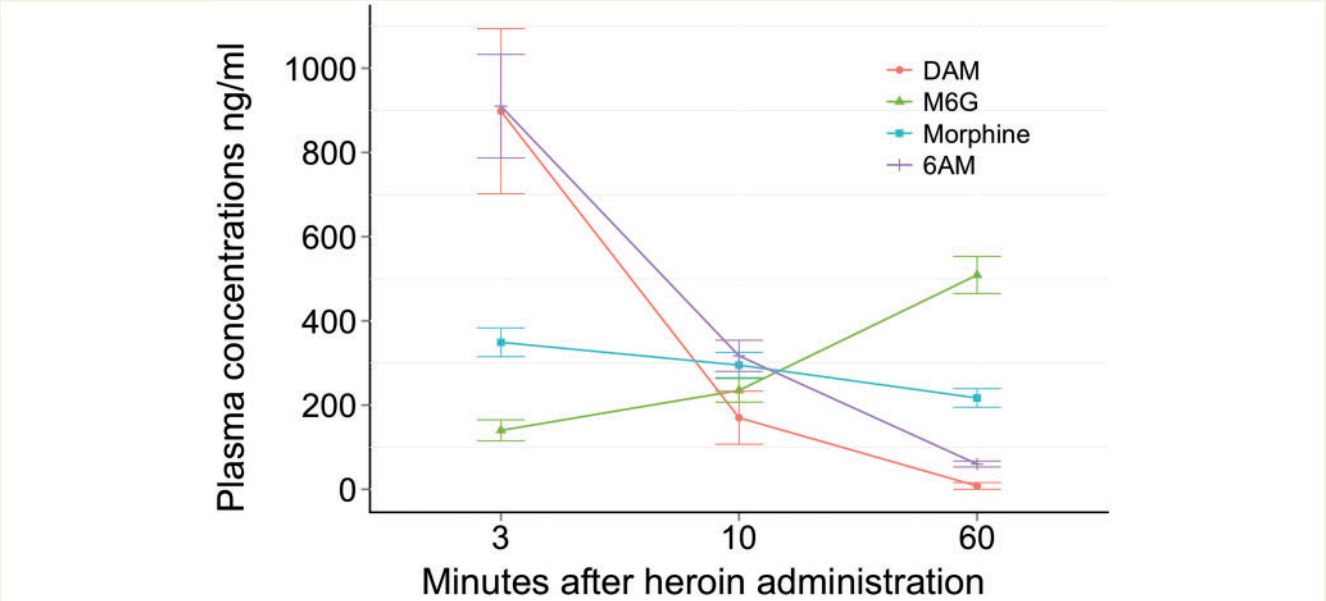


**Figure 4 Plasma concentrations after acute heroin treatment.** Plasma concentrations (mean ± SE) of heroin (diacetylmorphine, DAM), morphine, 6-acetylmorphine (6AM) and morphine-6-β-D-glucuronide (M6G) 3, 10 and 60 min after heroin administration.

diacetylmorphine, decreasing from 910 ng/ml (SD: 549) to 317 ng/ml (SD: 167) and 60 ng/ml (SD: 33) at 3, 10 and 60 min, respectively. In contrast, the mean concentrations of morphine at 3, 10 and 60 min after heroin injection were 349 ng/ml (SD: 140), 295 ng/ml (SD: 122), and 217 ng/ml (SD: 92), respectively. Morphine-6-β-D-glucuronide plasma concentrations steadily increased over the study period, from 140 ng/ml (SD: 25) to 235 ng/ml (SD: 28) and 509 ng/ml (SD: 44) at 3, 10 and 60 min, respectively. Plasma levels are depicted in Fig. 4.

## Psychophysiological stress responses

The results of stress hormone release have already been published (Schmidt *et al.*, 2014), but we report them here for the sake of completeness. Significantly lower levels of craving [$t(21) = 8.32$, $P = 0.0001$], withdrawal symptoms [$t(21) = 6.64$, $P = 0.0001$], state-anxiety [$t(21) = 6.17$, $P = 0.001$], as well as concentrations of serum cortisol [$t(21) = 4.95$, $P = 0.0001$], saliva cortisol [$t(21) = 4.47$, $P = 0.0002$] and adrenocorticotropic hormone [$t(21) = 5.17$, $P = 0.001$] were observed after heroin relative to the placebo treatment in patients. Healthy controls showed significantly lower anxiety scores than patients under placebo [$F(1,37) = 5.38$, $P = 0.0001$], whereas no difference was found in patients after heroin administration [$F(1,37) = 3.55$, $P = 0.072$]. Compared with healthy controls, patients exhibited significantly higher levels of adrenocorticotropic hormone [$F(1,37) = 16.80$, $P = 0.001$], serum cortisol [$F(1,37) = 1.02$, $P = 0.0027$], and salivary cortisol [$F(1,37) = 4.52$, $P = 0.001$] after placebo treatment, whereas patients under heroin showed significantly lower levels of adrenocorticotropic hormone [$F(1,37) = 0.004$, $P = 0.0001$] but not of serum cortisol [$F(1,37) = 5.41$, $P = 0.694$] or salivary cortisol [$F(1,37) = 2.65$, $P = 0.831$]. These parameters were not related to the results for plasma levels.

## Relation between amygdala connectivity and stress responses

Across patients, we found significant positive correlations between the left fusiform gyrus→amygdala connectivity and levels of state-anxiety ($r = 0.415$, $P = 0.005$, corrected), craving ($r = 0.373$, $P = 0.013$, uncorrected), withdrawal symptoms ($r = 0.450$, $P = 0.002$, corrected), serum cortisol ($r = 0.309$, $P = 0.042$, uncorrected) and adrenocorticotropic hormone ($r = 0.468$, $P = 0.001$, corrected) (Fig. 5A). The right amygdala→OFC connectivity also correlated positively with levels of craving ($r = 0.418$, $P = 0.005$, uncorrected) (Fig. 5B).

No relationships were found between connectivity estimates and plasma levels.

# Discussion

This paper presents a model-based investigation of amygdala-related connectivity that underlies the emotional regulation effect of heroin substitution in dependent patients. Our findings showed that stress-related placebo treatment in patients was accompanied by significantly increased fear-induced modulation of left fusiform gyrus→amygdala and right amygdala→OFC connectivity. Critically, heroin administration reduced both of these amygdala-related connectivity strengths to a level that did not differ from those

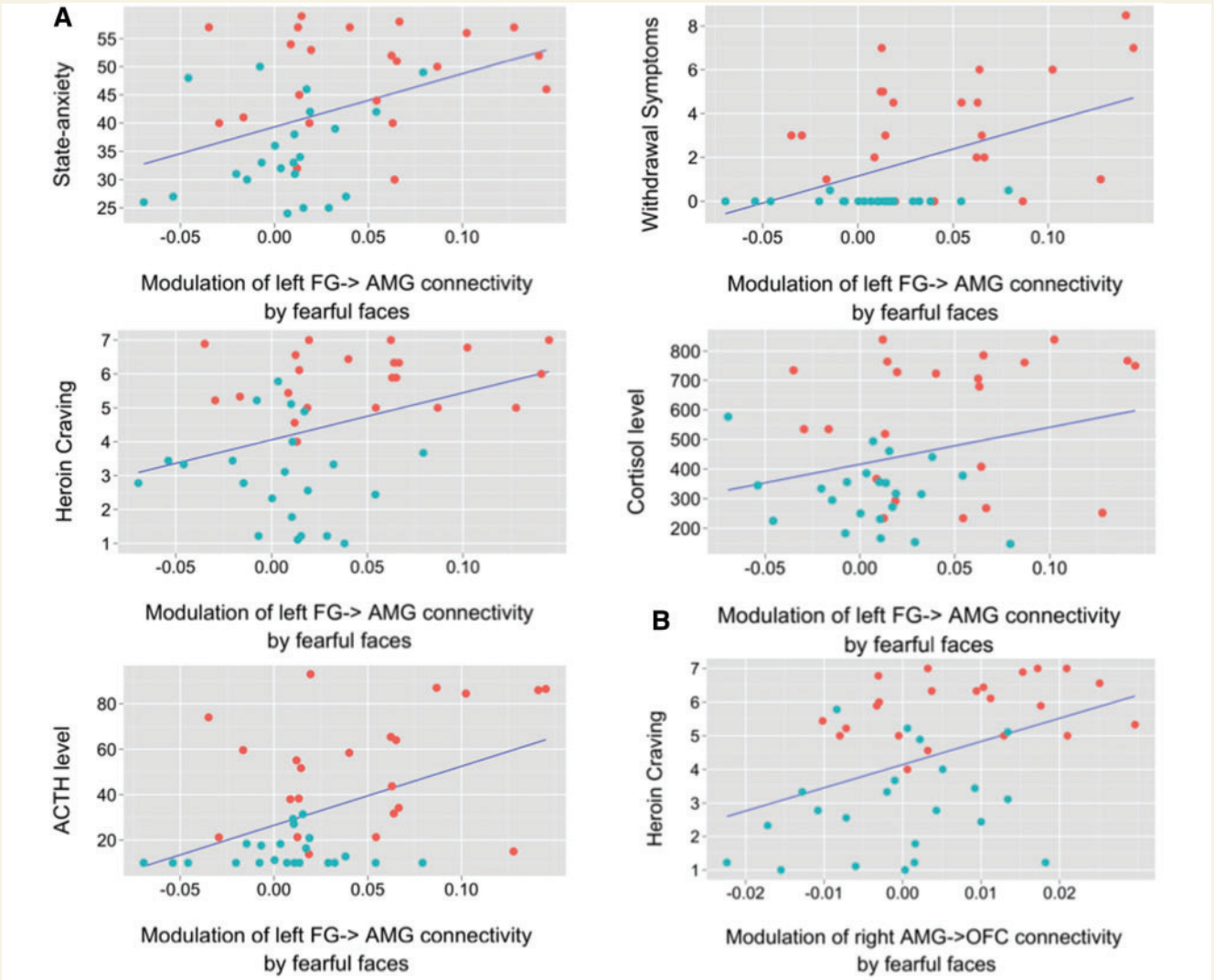


**Figure 5 Relation between modulation of connectivity by fearful faces and psychophysiological stress responses.** Significant positive correlations between the modulatory effect induced by fearful faces and stress responses across patients. (**A**) Correlation between left fusiform gyrus→amygdala connectivity and levels of state-anxiety (*top left;* r = 0.415, *P* = 0.005, corrected), craving (*middle left;* r = 0.373, *P* = 0.013, uncorrected), withdrawal symptoms (*top right;* r = 0.450, *P* = 0.002, corrected), adrenocorticotropic hormone (*bottom left;* ACTH, r = 0.468, *P* = 0.001, corrected) and cortisol (*middle right;* r = 0.309, *P* = 0.042, uncorrected). (**B**) Correlation between amygdala→OFC connectivity and levels of craving (r = 0.418, *P* = 0.005, uncorrected). FG = fusiform gyrus. Red dots represent the placebo treatment, while blue dots represent the heroin treatment.

of healthy controls. Given that corticotropin-releasing factors are recruited in the amygdala during the stage of withdrawal (Koob and Kreek, 2007; Koob, 2013), as reflected here by the placebo condition, the heroin-induced reduction of amygdala-related connectivity relative to the placebo treatment was probably driven by the attenuation of stress hormone release after heroin administration in these patients (Walter *et al.*, 2013). Indeed, we found a positive relation between fusiform gyrus→amygdala connectivity, state-anxiety, withdrawal symptoms and stress hormone release across all patients, which supports the biological realism of our model.

Our connectivity findings add to recent evidence that the left amygdala response to fearful faces correlates significantly with psychophysiological stress responses in heroin-dependent patients (Schmidt *et al.*, 2014). The present analyses suggest a more fine-grained mechanistic interpretation of this relationship, namely that the increased left amygdala activity during acute heroin withdrawal (placebo treatment) may be the result of increased input from the fusiform gyrus induced by fearful faces. This finding of pronounced fear-induced visual-limbic interplay in the anxious withdrawal state corresponds with the increased connectivity between the fusiform gyrus and amygdala during the resting state in other psychiatric conditions, such as social anxiety disorder (Liao *et al.*, 2010). In accordance with this, functional connectivity analyses have shown that visual-limbic coupling during fearful

face processing can be increased by psychosocial stress (Li *et al.*, 2014). Furthermore, a recent study showed that non-relapse participants exhibited reduced resting state functional connectivity between the amygdala and visual processing regions compared to controls and relapsed cocaine-addicted participants, which suggests that this may be a marker of early relapse risk (McHugh *et al.*, 2014). Our results thus suggest that the stress-related state during acute heroin withdrawal is accompanied by increased fear-induced modulation of visual-limbic connectivity. Crucially, heroin injection normalized this connectivity, as no difference from healthy controls was observed, which may point to a negative reinforcement mechanism at the neural system level.

Furthermore, relative to the placebo treatment in patients, heroin injection also reduced the right amygdala–OFC connectivity, giving a level comparable to that observed in healthy controls. It is interesting that the degree of amygdala→OFC connectivity was positively related to patients' craving behaviour. This result is consistent with evidence demonstrating functional connectivity between the OFC and amygdala during withdrawal-induced craving (Koob and Volkow, 2010). The contribution of the OFC to heroin craving has further been demonstrated in a previous PET study (Sell *et al.*, 2000). The OFC seems to be important for stress-induced reinstatement of drug-seeking, i.e. it drives the motivation for new drug intake (Schoenbaum and Shaham, 2008), during which it is reciprocally connected with the amygdala (Lasseter *et al.*, 2009). Therefore, the finding of increased amygdala→OFC connectivity during acute heroin withdrawal may indicate that there is both stress-induced increase in heroin craving and resulting reinforced motivation for renewed heroin intake. This stress-induced compulsion to consume the drug was normalized by acute heroin administration, as indicated by reduced amygdala–OFC connectivity. In contrast to this acute heroin-induced normalization of OFC–amygdala connectivity in heroin-dependent patients, a previous investigation in methadone-maintained patients showed that the OFC still remained high in response to heroin-related cues after the daily methadone dose (Langleben *et al.*, 2008), which suggested that methadone maintenance patients remain at risk for relapse into illicit heroin use. Thus, in accordance with data supporting the superiority of heroin compared to methadone maintenance in severely opioid-dependent subjects, especially if treatment is maintained for 12 months or longer (van den Brink *et al.*, 2003; Oviedo-Joekes *et al.*, 2009), heroin-induced normalization of OFC connectivity may reflect a benefit compared to methadone maintenance treatment in preventing relapses to self-administrations in prone patients. However, this is clearly a speculative observation at the present time and it is important to emphasize that we do not wish to make any claims here for an advantage of heroin maintenance therapy over methadone maintenance therapy. The results from the two studies are not directly comparable, owing to a number of major methodological differences, including different measurement protocols and fundamentally different paradigms. For instance, Langleben *et al.* (2008) measured functional MRI responses to heroin-related cues pre- and post-treatment, whereas we used a fearful face task to assess stress responses after heroin and placebo administration.

There are important limitations to our studies. Effective connectivity was computed using a fairly simplistic neuronal face network, without considering, for example, the precuneus or a more detailed partitioning of visual face-sensitive processing areas, as done in previous DCM studies (Fairhall and Ishai, 2007; Dima *et al.*, 2011). However, given the impact of stress, craving and anxiety on visual-limbic and prefrontal-limbic connections (Koob and Volkow, 2010; Frick *et al.*, 2013; Fan *et al.*, 2014; Li *et al.*, 2014) and that heroin acutely reduced levels of stress hormones, withdrawal, craving and anxiety (Walter *et al.*, 2013), we were explicitly interested in how heroin acutely modulated fear-induced modulation of fusiform gyrus-amygdala and amygdala–OFC connectivity. Nevertheless, including more visual regions implicated in face processing—such as the inferior occipital gyrus—may influence how information enters into the fusiform gyrus and propagates further across the network. In this respect, there is evidence that fusiform gyrus activity decreases with increasing repetitions of faces (Reber *et al.*, 2005), which is perhaps reflected by the negative driving inputs into the fusiform gyrus in this study. Repeated exposure to identical emotional faces also modulates fusiform gyrus-amygdala connectivity (Herrington *et al.*, 2011). It is therefore important in further studies to address how such priming effects evolve across different visual processing areas and propagate further to the limbic and prefrontal system. Furthermore, because of the cross-sectional design of this study, we were not able to infer how this acute heroin effect on amygdala-related connectivity developed over the duration of the maintenance therapy and how long it lasted. This should be addressed in longitudinal studies and compared with long-acting steady-state managements, such as methadone maintenance therapies using adequate methadone dosing levels, given that high doses of methadone were more effective than low doses in the reduction of illicit opioid use (Strain *et al.*, 1999; Farré *et al.*, 2002). A distinction must be drawn between the placebo-induced state of withdrawal in the current study and the withdrawal state in active drug users. Although the experimentally induced increase in withdrawal signs after placebo treatment allowed us to study negative reinforcement mechanisms, this approach also impedes clinical inferences. Therefore, further analyses of this sort are needed in various levels of withdrawal, as well as before and after substitution treatments. Expectation effects might have influenced our findings, given that healthy controls were aware that they were going to receive a placebo treatment only. Finally, we cannot exclude the possibility that some of the patients had a history of psychiatric disorder, including other comorbid conditions, which is a potential confounding factor.

In conclusion, our findings extend previous evidence for hyperactive amygdala-related network connectivity in abstinent heroin addicts (Xie *et al.*, 2011) and suggest that the stressful state during acute heroin withdrawal is associated with increased amygdala-related connectivity during fearful face processing. The results further indicate that a single dose of heroin to heroin-maintained patients transiently normalizes amygdala-related brain connectivity during fearful face processing. More research with follow-up measurements is required to quantify whether the recording of amygdala-related connectivity during fearful face processing may provide a prognostic tool to assess stress levels in heroin-dependent patients before and after maintenance treatments.

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## Supplementary material

Supplementary material is available at *Brain* online.

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