Abnormal functional integration of thalamic low frequency oscillation in the BOLD signal after acute heroin treatment

Abbreviated title: Heroin effects on thalamic resting state activity

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ABSTRACT

Heroin addiction is a severe relapsing brain disorder associated with impaired cognitive control, including deficits in attention allocation. The thalamus has a high density of opiate receptors and is critically involved in orchestrating cortical activity during cognitive control. However, there have been no studies on how acute heroin treatment modulates thalamic activity. In a cross-over, double-blind, vehicle-controlled study, 29 heroin-maintained outpatients were studied after heroin and placebo administration, while 20 healthy controls were included for the placebo condition only. Resting-state functional magnetic resonance imaging was used to analyse functional integration of the thalamus by three different resting state analysis techniques. Thalamocortical functional connectivity (FC) was analysed by seed-based correlation, while intrinsic thalamic oscillation was assessed by analysis of regional homogeneity (ReHo) and the fractional amplitude of low frequency fluctuations (fALFF). Relative to the placebo treatment and healthy controls, acute heroin administration reduced thalamocortical FC to cortical regions, including the frontal cortex, while the reductions in FC to the mediofrontal cortex, orbitofrontal cortex and frontal pole were positively correlated with the plasma level of morphine, the main psychoactive metabolite of heroin. Furthermore, heroin treatment was associated with increased thalamic ReHo and fALFF values, whereas fALFF following heroin exposure correlated negatively with scores of attentional control. The heroin-associated increase in fALFF was mainly dominated by slow-4 (0.027-0.073 Hz) oscillations. Our findings show that there are acute effects of heroin within the thalamocortical system and may shed new light on the role of the thalamus in cognitive control in heroin addiction. Future research is needed to determine the underlying physiological mechanisms and their role in heroin addiction.

Keywords: heroin addiction; heroin effects; thalamus; cognitive control; resting state; functional connectivity

INTRODUCTION

Loss of cognitive control, including compromised ability to inhibit impulsive drug-driven behaviour, is a key hallmark of drug addiction (Perry and Carroll, 2008). For example, it has been shown that heroin addicts have a significantly lower degree of impulse control than normal controls (Lee and Pau, 2002) and have difficulties in shifting attention from one subject to another (Ornstein, et al., 2000). Such deficits in selective attention and impulsivity are common in opiate-dependent patients (Bracken, et al., 2012; King, et al., 1999), and even persist in abstinence after stopping a maintenance treatment (Prosser, et al., 2009). On the other hand, regular maintenance treatment may improve cognitive performance in heroin addicts, including attention and executive function (Wang, et al., 2014).

Neuroimaging studies have indicated that impaired cognitive control in heroin addicts may arise from abnormal prefrontal brain activity (Pau, et al., 2002), in particular from the inferior frontal gyrus (IFG) (Fu, et al., 2008a; Lee, et al., 2005). Besides prefrontal brain regions, the thalamus is critically implicated in cognitive control and plays a core role in processing and maintaining attention (de Bourbon-Teles, et al., 2014; Lawrence, et al., 2003). There is evidence that the effects of heroin are mostly mediated by μ receptors, which are densest in the cingulate gyrus, ventral tegmental area, cerebellum, thalamus, and hypothalamus (Maurer, et al., 1983; Pfeiffer, et al., 1982). Within the thalamus, μ receptors are densest within the dorsomedial, lateroposterior and laterodorsal thalamic nucleus, as shown by in situ hybridisation in post-mortem studies of humans (Peckys and Landwehrmeyer, 1999). Given the high density of opiate receptors in the thalamus (Apkarian, et al., 2005; Lever, 2007), the reduced glucose metabolism in patients in a methadone maintenance program (Galynker, et al., 2000; Prosser, et al., 2009) and the decreased regional homogeneity in the resting state thalamus (ReHo) in chronic heroin addicts (Qiu, et al., 2011), it is conceivable that acute heroin use is associated with abnormal thalamic activity.

However, it is also becoming increasingly apparent that complex psychological processes, such as cognitive control, result from interactions between different brain regions rather than from local activity per se. Within this perspective, the thalamocortical system has an important role in synchronising the activities of thalamic and cortical neurons and is thus essential in orchestrating and integrating function across different brain regions (Jones, 2002; Steriade, 2006). A critical point is that the thalamus and cortical areas are functionally connected during cognitive control (Aron and Poldrack, 2006). This evidence suggests that impaired cognitive control in heroin addiction might result not only from abnormal task-associated activity in the IFG (Fu, et al., 2008a; Lee, et al., 2005), but also from disrupted thalamocortical connectivity.

We have now carried out a cross-over, double-blind and vehicle-controlled study in a clinical sample of heroin-maintained patients, in which we used resting state functional magnetic resonance imaging (fMRI) to explore how heroin acutely modulates the functional integration of the thalamic system. Resting state fMRI provides a useful tool to study functional integration of brain regions, at the level of large scale neural systems, by analysis of low frequency fluctuations in the blood oxygen level-dependent (BOLD) signal (Friston, 2009). We computed thalamocortical functional connectivity (FC) using a seed-based correlation approach (Biswal, et al., 1995), whereas local thalamic low frequency oscillation characteristics were assessed by analysis of ReHo and the fractional amplitude of low frequency fluctuations (fALFF). We expected that acute heroin administration would modulate thalamic functional integration during resting state oscillation.

METHODS

Subjects

This study was carried out with two group of subjects. 29 heroin-maintained outpatients (19 male) were recruited from the Centre of Substance Use Disorders of the Psychiatric University Hospital of Basel. Inclusion criteria were age older than 18 years, past history of illicit intravenous heroin consumption, and participation in the heroin-assisted treatment for at least 6 months with an unchanged heroin dose during the previous 3 months. In addition, 20 healthy controls were recruited from the general population in the same geographical area as the patients. Both subject groups were carefully screened, using a semi-structured clinical interview. The exclusion criteria were a positive alcohol breathalyser test, an additional physical disease, or a comorbid psychiatric disorder. Controls and patients were told to abstain from illicit drug consumption for the duration of the study, as well as to abstain from alcohol intake for 72 hours and from smoking for 2 hours. Nevertheless, 15 patients were tested positive for cocaine and 9 patients and 5 healthy controls for cannabis at one or both points of the measurements. The subjects' characteristics are summarised in Table 1.

The study was approved by the local ethics committee and registered with http://clinicaltrials.gov (ID NCT01174927). After receiving a written and oral description of the aim of this study, all participants gave written informed consent statements before inclusion.

Experimental design

The study was performed using a cross-over, double-blind and placebo-controlled design with two temporally distinct examination sessions; there was high overlap in used patients with previously published studies which resulted from the same examination (the procedure and healthy controls were always the same) (Schmidt, et al., 2014; Schmidt, et al., 2013a;

Schmidt, et al., 2013b; Schmidt e, et al., 2015; Walter, et al., 2014). All studies derived from theHeroin (Diaphin®) and placebo (saline solution) were administered intravenously through an indwelling intravenous catheter over a period of 30 seconds, as recently described (Walter, et al., 2014). During both sessions (with a time interval of about two weeks), patients received both heroin and placebo. The subjects who received heroin before scanning were administered vehicle after scanning (i.e., 60 min after the first injection), whereas the subjects who received placebo before scanning were administered heroin after scanning. The order was randomised in a balanced manner and did not affect the findings obtained. The administered heroin dose was equivalent to the subjects' individual dose. Healthy controls received only a placebo administration and were examined only once.

Bioanalytical and behavioural measurements

Data on plasma levels have recently been published elsewhere (Walter, et al., 2014; Walter, et al., 2013). As we used the mean peak levels for later correlation analyses, we provide a brief summary of the analytical method. Plasma levels of diacetylmorphine (heroin) and morphine were quantified in venous ammonium heparinised plasma by 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. The sample preparation and instrumental conditions were as previously described in detail (Bourquin D, et al., 1999). Plasma drug levels were measured three times, at 3, 10 and 60 minutes after drug injection. Individual peak concentrations of diacetylmorphine and morphine (the main psychoactive metabolite of diacetylmorphine) were used for further correlation analysis.

Attention was assessed using the 30-item self-report questionnaire of the Barratt Impulsiveness Scale (BIS) (Patton, et al., 1995). Healthy controls and patients completed the questionnaire after receiving the treatment and before the scan. Patients completed the questionnaire in

both examination sessions. The BIS questionnaire assesses 6 first order and 3 second order factors of impulsivity, including attention, motor, and non-planning categories, as well as a total impulsivity score. Given the key role of the thalamus in attentional control (de Bourbon-Teles, et al., 2014), only first and second order attention scores were used for subsequent correlation analyses. The values of the first order attention scores range from 5 to 20, where a higher score implies worse attentional control. The second order attention score includes the first order score and scores for cognitive instability, with a range from 8 to 32.

Image acquisition

Scanning was performed on a 3 Tesla scanner (Magnetom Verio, Siemens Healthcare, Erlangen, Germany). A 5 minutes resting state condition (20 minutes after substance administration) was examined with a whole brain echo planar imaging (EPI) sequence (TR = 2000 ms, TE = 28 ms, flip angle = 82°, field of view = 228×228 mm^2, 32 slices, voxel size = 3.6×3.6×3.8 mm^3). In total, 152 EPI volumes were acquired. In addition, images were acquired with a high resolution T_1-weighted magnetisation-prepared rapid acquisition gradient echo (MPRAGE) image (TR = 2000 ms; TE = 3.37 ms; flip angle = 8°; inversion time = 1000 ms; 176 slices; slice thickness = 1 mm; voxel size = 1×1×1 mm^3).

Image preprocessing

EPI volumes were preprocessed using statistical parametric mapping software (SPM8; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). EPI volumes of each scanning session were realigned using rigid transformation, normalised by linear and nonlinear transformation into MNI space (Montreal Neurological Institute), by using the ICBM152 template (International Consortium for Brain Mapping), spatially smoothed with a 6 mm full width half maximum (FWHM) Gaussian kernel, and band-pass filtered for the frequency range of 0.01-0.08 Hz.

Thalamocortical connectivity (seed-based correlation)

Functional data were analysed using the seed-driven approach of the CONN toolbox (CONN 13.1; http://web.mit.edu/swg/software.htm) (Whitfield-Gabrieli and Nieto-Castanon, 2012). We defined the bilateral thalamus as seed region of interest for the between-group (patients and controls) and the between-treatment (placebo and heroin) analyses. In order to understand between-treatment differences in more detail, we additionally performed analyses with seeds of the bilateral medial dorsal, lateral posterior and lateral dorsal thalamic nucleus, which are known to show high μ receptor density (Peckys and Landwehrmeyer, 1999). The masks were generated by WFU PickAtlas (Maldjian, et al., 2003). Preprocessed and band-pass-filtered EPI volumes were used for computation of seed-to-voxel FC maps. The correlation coefficients between the signal within the thalamic seed and the signal of all other brain voxels were estimated. Confounding signals related to white matter and cerebrospinal fluid were removed through linear regression. Realignment parameters (six dimensions with first order derivative) were defined as first level covariates.

Thalamic regional homogeneity (ReHo)

For each subject and treatment, ReHo maps were computed using Kendall's coefficient of concordance (KCC), as integrated in the REST toolkit (REST 1.8; http://www.restfmri.net) (Song, et al., 2011). The BOLD time course in preprocessed normalised non-smoothed EPI volumes was corrected for linear trends and a temporal band-pass filter of 0.01-0.08 Hz was applied. KCC was calculated for each voxel time series, in order to measure the similarity of the ranked time series with its 26 neighbour voxels. The resulting KCC-ReHo maps were standardised by dividing each voxel by the global mean within a whole brain mask and smoothed with a 6 mm FWHM Gaussian kernel.

Thalamic fractional amplitude of low frequency fluctuations (fALFF)

Preprocessed normalised and non-smoothed EPI volumes were used to compute fALFF maps with the REST toolkit (Song, et al., 2011). The frequency-filtered time series of each voxel are described by a Fourier series, where the BOLD signal is expressed as a sum of sines and cosines (Davis, 1989). The ALFF value is the averaged square root of the power spectrum obtained via fast Fourier transformation, whereas fALFF values reflect the ratio of the power spectrum of the signal (0.01-0.08 Hz) to that of the entire frequency range (Zou, et al., 2008). As categorised in previous research, we also compared thalamic frequency between heroin and placebo treatment by preprocessing and reanalysing data using slow-4 (0.027-0.073 Hz) and slow-5 (0.01-0.027 Hz) frequency bands (Buzsaki and Draguhn, 2004; Zhang, et al., 2015; Zou, et al., 2008). The computed fALFF maps were standardised by dividing each voxel by the global mean within a whole brain mask and smoothed with a 6 mm FWHM Gaussian kernel.

Computation of treatment difference maps

We used the image calculation function implemented in SPM8 to compute difference maps between the placebo and heroin treatment. Treatment-related changes in thalamocortical FC (magnitude of correlation coefficient) were computed by subtracting the placebo from the heroin treatment. To investigate only LFF values of positive functional connectivity we decide to exclude all negative values within the placebo and heroin treatment. Treatment-associated differences in ReHo and fALFF values were computed by subtracting heroin from placebo maps.

Statistical analysis

Between-group and between-treatment analyses were performed using a general linear model (GLM) in SPM8. Seed-based thalamic FC, ReHo and fALFF maps were tested by voxelwise whole brain analysis. Paired t tests were used to test between-treatment differences in patients

and two sample t tests were used to test differences between patients and healthy controls. Treatment difference maps of thalamocortical FC, ReHo and fALFF were used for whole brain correlation analyses with peak plasma levels of morphine. First and second order BIS attention scores were used separately for the correlation analysis with thalamocortical FC, ReHo and fALFF maps after the placebo and heroin administration in heroin-dependent patients. All analyses were performed using a cluster-level inference strategy with a cluster-forming threshold of $p < 0.001$ and adjusted for family-wise error (FWE) at $p < 0.05$ (Hayasaka, et al., 2004). For correlation analyses, we used a binary mask of main treatment differences with a cluster-forming threshold of $p < 0.05$ and adjusted for family-wise error (FWE) at $p < 0.05$. Statistical comparisons of demographic and clinical characteristics were performed with SPSS 19 (IBM SPSS Statistics; Armonk, NY: IBM Corp) using independent sample t tests for parametric and χ^2 tests for nonparametric data.

RESULTS

Demographic and clinical characteristics

The groups of patients and controls did not differ in age, gender or cannabis use. Patients consumed significantly more cocaine, more cigarettes per day and were less often employed than controls. The demographic and clinical characteristics of the study sample are summarised in Table 1.

Bioanalytical and behavioural measurements

The mean plasma peak level of diacetylmorphine was 1042.4 ng/ml and ranged from 40 to 3051 ng/ml. Morphine concentrations showed a mean peak of 976.6 ng/ml and ranged from 39 to 3885 ng/ml. The results on drug plasma levels and their temporal decay have been previously described in more detail (Walter, et al., 2014). Peak plasma levels of diacetylmorphine ($p = 0.343$, $r = 0.203$) and morphine ($p = 0.863$, $r = -0.037$) did not correlate significantly with daily heroin dose.

No significant differences in BIS total score ($p = 0.209$, $t = 1.286$), or first order ($p = 0.332$, $t = 0.987$) or second order attention scores ($p = 0.124$, $t = 1.586$) were found after heroin and placebo treatment in patients. Relative to healthy controls, patients after heroin treatment showed significantly higher BIS total scores ($p = 0.013$, $t = 2.568$), and first order ($p = 0.001$, $t = 4.164$), and second order attention scores ($p = 0.011$, $t = 2.636$) (Table 1). After placebo treatment, patients showed significantly higher BIS total scores ($p = 0\text{-}003$, $t = 3.115$), first order ($p < 0.001$, $t = 4.079$), and second order attention scores ($p = 0.002$, $t = 3.246$) in comparison to healthy controls.

Thalamocortical connectivity (seed-based correlation analysis)

In comparison to placebo, heroin in patients reduced thalamic FC to the bi-hemispherical frontal, parietal and temporal regions and increased thalamus FC (Figure 1, Table 2). Heroin also reduced thalamic FC to parietal, temporal and occipital regions relative to healthy controls, whereas no heroin-induced increase was found. After placebo administration, patients showed reduced thalamic FC to the temporal gyrus compared with healthy controls.

Analyses of FC of specific thalamic nuclei in patients revealed a heroin-associated reduction in connectivity strength between the precentral gyrus and the lateral dorsal nucleus. The lateral posterior nucleus was instead associated with increased connectivity strength within the thalamus during heroin treatment. The medial dorsal nucleus was associated with the broadest heroin-associated reduction in connectivity strength, including the temporal, parietal, occipital and frontal lobe (Table 3).

Thalamic regional homogeneity (ReHo)

Between-treatment analysis of ReHo revealed that heroin treatment in patients significantly increased values in the bilateral thalamus, posterior cingulate, postcentral and precentral gyrus, compared with the placebo treatment (Figure 3, Table 4). In comparison to heroin treatment, increased ReHo values were found during placebo in the temporal, frontal and occipital cortex. In comparison to heroin treatment, healthy controls showed increased ReHo values in the frontal cortex.

Thalamic fractional amplitude of low frequency fluctuations (fALFF)

Between-treatment analysis of fALFF in patients revealed increased values for heroin treatment within the precentral gyrus and the thalamus relative to the placebo treatment (Figure 32 Table 5). Healthy controls showed increased fALFF values in the precuneus and posterior cingulate gyrus and decreased fALFF values in the cerebellum, midbrain and amygdala in

comparison to the heroin treatment. Placebo treatment in patients was associated with increased fALFF values in the cerebellum, inferior temporal and parahippocampal gyrus.

In depth analysis of slow-4 and slow-5 frequency bands within patients revealed increased slow-5 fALFF values in the precentral gyrus during heroin treatment. Slow-4 fALFF was associated with increased values in the bilateral thalamus during heroin treatment (Table 6).

Correlation analyses

Correlation analyses showed that peak plasma levels of morphine correlated positively with heroin-induced reduction in thalamic FC to the frontal orbital cortex, mediofrontal cortex and frontal pole (Table 7). By analysing heroin and placebo treatment separately, we found a negative correlation between first-order attention score of BIS (heroin treatment) and fALFF values (heroin treatment), within a cluster including the thalamus, midbrain and hypothalamus (Table 8, Figure 3). No correlation was found between first and second order attention scores of BIS and treatment-dependent thalamocortical FC and ReHo.

DISCUSSION

In this study, we investigated whether acute heroin treatment leads to alterations in spontaneous neural activity within the thalamocortical system. We performed three different resting state analyses to detect modulations of functional integration and segregation of thalamic low frequency oscillation and examined whether these effects were related to clinical characteristics and behavioural indices of attention. We could show that, in comparison to placebo treatment and healthy controls, heroin acutely reduced thalamocortical FC to multiple cortical regions, including parts of the frontal cortex. The heroin-induced reductions in FC from the thalamus to the mediofrontal cortex, orbitofrontal cortex and frontal pole were positively correlated with plasma levels of morphine. Furthermore, and in contrast to reductions in thalamic FC, we found increased thalamic ReHo and fALFF values during heroin treatment. The heroin-associated increase in fALFF values were driven by alterations in the slow-4 oscillation band. Plasma levels of heroin were positively correlated with treatment differences in fALFF in the bilateral lateral occipital cortex, while fALFF within the right thalamus correlated negatively with attention scores measured by BIS.

Impaired cognitive control plays an important role in the compulsive and drug-seeking behaviour of drug-dependent subjects (Perry and Carroll, 2008). It has repeatedly been shown that this deficit in cognitive functioning in heroin addicted individuals is accompanied by reduced activity in the right IFG and ACC (Fu, et al., 2008b; Lee, et al., 2005). Our group has recently extended these findings by showing that acute heroin administration not only impairs cognitive control by reducing IFG and ACC activity in heroin-dependent patients, but also effective connectivity from the ACC to the IFG during a cued Go/No-Go task (Schmidt, et al., 2014; Schmidt, et al., 2013b). However, besides the crucial interplay between the dorsal ACC and IFG, the IFG is also functionally connected with the thalamus during cognitive functioning

(i.e. response inhibition) (Aron and Poldrack, 2006). In the present study, we found that acute heroin treatment reduced FC between the thalamus and frontal brain regions, which suggests that these reductions may modulate cognitive functioning in heroin-dependent subjects. This finding adds to our previous results that acute heroin administration not only reduces ACC activity and its connectivity to the IFG (Schmidt, et al., 2014; Schmidt, et al., 2013a), but also the connectivity from the thalamus to the frontal cortex. The reduced thalamocortical FC after heroin treatment may be regarded as desynchronised activity between the thalamus and the cortex and may contribute to impairment in the alerting attention network. Heroin-associated reduction in frontal perfusion and gray matter volume, as shown in our previous studies (Denier, et al., 2013a; Denier, et al., 2013b), may be associated with reduction in thalamofrontal FC.

In contrast to the heroin-induced reduction in thalamocortical FC, we found enhancement of local thalamic parameters, including fALFF and ReHo values. While resting state FC can be taken as a measure of coupling in oscillation between distant regions, ALFF/fALFF measures reflect the extent of BOLD-associated spontaneous oscillation and so indirectly its neural activity (Duff, et al., 2008; Fransson, 2006). ReHo is a good index of local integrity as assessed by oscillation synchronisation (Zang, et al., 2004). However, the direct relationships of the three parameters, FC, fALFF and ReHo, are not yet fully understood. There are only two studies examining long term ALFF changes in heroin addicts, but no ALFF changes were found in the thalamus. Compared to healthy controls, heroin addicts showed decreased ALFF values in the temporal and frontal lobe, including the orbitofrontal cortex and the ACC. Moreover, heroin addicts showed decreased ALFF in the right caudate that correlated with the duration of heroin use (Wang, et al., 2013). A previous study in heroin-dependent individuals showed decreased ReHo values in the thalamus relative to healthy controls (Qiu, et al., 2011). This discrepancy may result from differences in the included patient samples, such as differences in the durations of heroin use, duration of maintenance treatment or dose of maintenance

treatment. Furthermore, the placebo treatment in our study may induced a state of heroin withdrawal (Schmidt, et al., 2013a), resulting in a different psychological state from that of the patients in Qiu et al. (2011). However, despite this discrepancy, the authors concluded that this decrease in the ReHo values in the bilateral medial dorsal nucleus might mediate the attention deficits in chronic heroin users. In accordance with such an interpretation, we could show that fALFF values in the right thalamus after heroin treatment were negatively correlated with subjective scores on attentional control. This corresponds with a previous study showing a negative correlation between attentional BIS scores in codeine-dependent subjects and ReHo values in the thalamus (Qiu, et al., 2013) as ReHo and fALFF values are known to be correlated. More work is needed to understand the relation between thalamic ReHo and ALFF alteration and attentional control.

Opioid-associated alterations in thalamic functionality are well documented in animals. In particular, Brunton and Charpak showed that a systemically administered μ-opioid agonist inhibited the entire thalamus by inducing hyperpolarisation and shifting thalamic cell firing from the tonic to the bursting mode (Brunton and Charpak, 1998). The burst firing mode is characteristic of thalamic relay neurons (Jahnsen and Llinas, 1984) and plays an important role in slow wave sleep and drowsiness (Livingstone and Hubel, 1981; Steriade, et al., 1993). We may speculate that our findings of heroin-induced reduction in thalamocortical FC and enhancement of fALFF and ReHo reflect a shift towards the thalamic burst firing mode on a higher functional level.

Some limitations need to be considered. Our patients were recruited from a population which mainly consisted of individuals with long standing polysubstance use, including cocaine consumption. Although this problem is virtually inevitable when chronic heroin-dependent individuals are examined, cocaine and other drug use may have confounded our findings. However, the greatest differences were found between heroin and placebo treatment within patients.

Another limitation is that BIS is not a direct psychometric measurement of attention and impulsivity. Therefore the BIS scores are stable within patients, did not differ between treatments, but may be substantially influenced by recurring heroin effects. A more direct cognitive assessment of attention and impulsivity would help to clarify the acute modulation of heroin on attentional control functions and whether this effect is related to the reported alterations in thalamic activities.

In conclusion, our results showed that acute heroin administration leads to abnormalities in functional integration and segregation of thalamic resting-state oscillation. Abnormalities were partially related to clinical characteristics and plasma levels of morphine. Reduced thalamocortical FC and altered intrinsic thalamic oscillation characteristics may explain some deficits in cognitive functioning in heroin-dependent patients. Further research is needed to elucidate the relationship between thalamic function and cognitive control in heroin addiction.

Acknowledgments

We would like to thank the staff of the heroin-assisted treatment in Basel (Janus) who helped us to conduct this study. In particular, we thank Jeannette Kaiser, Sandra Gropp, Eva Müller and Rainer Kreider.

Funding

This study was funded by the Swiss National Science Foundation (SNSF) (32003B-127544) (M.W., S.B., G.A.W., A.R-R.) and Freiwillige Akademische Gesellschaft Basel (A.S.). All authors declare that they have no conflicts of interest.

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Figure legends

Figure 1: Thalamocortical FC differences between heroin and placebo treatment in heroin-dependent patients. Red indicates increased FC by heroin and blue indicates reduced FC relative to the placebo treatment.

Figure 2: Significant positive relation of the heroin-induced reduction in thalamic FC to the left (128 voxels) and right orbitofrontal cortex (65 voxels) and the left frontal pole (173 voxels) with the peak plasma levels of morphine.

Figure 3: Significant increase in thalamic ReHo and fALFF values induced by acute heroin compared with placebo treatment. Spectral colours indicate mean thalamic ReHo and fALFF values of heroin and placebo treatment.

TABLES

Table 1: Socio-demographic and diagnostic characteristics of the study sample

Measurements	Patients (n = 29)	Healthy controls (n = 20)	Between-group statistics
Age (years), mean (SD)	41.5 (6.1)	40.3 (10.9)	T(48) = 0.506 p = 0.615
Gender (women/men)	8/21	6/14	$\bullet^2 = 0.034$ p = 0.854
Employment (yes/no)	13/16	20/0	$\bullet^2 = 16.385$ **p = 0.00005**
Daily heroin dose (mg/day), mean (SD)	323.5 (133.3)	-	-
Duration of dependence (years), mean (SD)	20.6 (7.2)	-	-
Age at the first-time heroin use (years), mean (SD)	18.3 (4.8)	-	-
Duration of opioid maintenance (years), mean (SD)	6.9 (4.5)	-	-
Number of cigarettes per day, mean (SD)	20.76 (9.0)	11.50 (8.2)	T(48) = 3.664 **p = 0.001**
Cannabis abuse, (yes/no)	9/20	5/15	$\bullet^2 = 0.211$ p = 0.646
Cocaine abuse, (yes/no)	15/14	0/20	$\bullet^2 = 14.909$ **p = 0.0001**
BIS total score, mean (SD)	65.93 (6.9)	61.15 (5.6)	T(47) = 2.568 **p = 0.013**
BIS first-order attention score, mean (SD)	11.24 (2.0)	9.10 (1.4)	T(47) = 4.164 **p = 0.0001**
BIS second-order attention score, mean (SD)	16.72 (3.0)	14.70 (2.1)	T(47) = 2.636 **p = 0.011**

BIS: Barrett Impulsiveness Scale; SD: Standard deviation.

Table 2: Resting-state associated thalamocortical functional connectivity (FC)

Brain region	Cluster size (voxels)	Cluster p-value (FWE corrected)	MNI coordinats (x y z)	Hemisphere	Z-value at voxel level
Heroin > Placebo Treatment					
Thalamus	174	0.007	-16 -16 6	L	5.07
			-2 -24 4	L	3.53
Thalamus	117	0.048	6 -14 4	R	3.94
			12 -20 12	R	3.61
Heroin < Placebo Treatment					
Precuneus	495	< 0.001	10 -52 34	R	4.68
			6 -62 28	R	4.05
			12 -52 42	R	4.04
Lateral Occipital Cortex	159	0.012	48 -66 26	R	4.29
Middle Temporal Gyrus	165	0.01	58 -6 -26	R	4.24
			60 -4 -12	R	4.00
			58 -12 -18	R	3.82
Precuneus	195	0.004	-8 -66 14	L	4.12
			-4 -54 14	L	3.42
Medial Frontal Gyrus	118	0.046	14 48 12	R	4.11
			6 54 8	R	3.50
			-2 50 10	L	3.44
Superior Parietal Lobule	119	0.045	-22 -54 66	L	4.04
Controls > Heroin Treatment					
Middle Temporal Gyrus	507	< 0.001	58 -6 -20	R	5.00
			50 2 -32	R	4.03
			64 -24 -20	R	3.91
Precentral Gyrus	162	0.025	16 -18 72	R	4.68
			14 -24 66	R	3.82
			10 -18 54	R	3.68
Lateral Occipital Cortex	349	< 0.001	50 -62 28	R	4.60
			56 -54 22	R	4.36
Middle Temporal Gyrus	156	0.03	-64 -8 -26	L	4.51
Precuneus	1380	< 0.001	0 -54 8	R	4.48
			18 -50 40	R	4.35
			14 -40 4	R	4.26
Controls < Heroin Treatment					
none					
Controls > Placebo Treatment					
Inferior Temporal Gyrus	237	0.004	-52 -30 -28	L	4.09
			-62 -26 -30	L	3.96
			-50 -40 -28	L	3.80
Controls < Placebo Treatment					
none					

FWE: family-wise error; L: left; R: right.

Table 3: Resting-state associated functional connectivity (FC)of specific thalamic nuclei.

Brain region	Cluster size (voxels)	Cluster p-value (FWE corrected)	MNI coordinats (x y z)	Hemisphere	Z-value at voxel level
Lateral Dorsal Nucleus: Heroin < Placebo Treatment					
Precentral Gyrus	141	0.019	-12 -26 80	L	4.90
Precentral Gyrus			-20 -24 78	L	4.04
Precentral Gyrus			-22 -18 72	L	3.27
Lateral Dorsal Nucleus: Heroin > Placebo Treatment					
none					
Lateral Posterior Nucleus: Heroin < Placebo Treatment					
none					
Lateral Posterior Nucleus: Heroin > Placebo Treatment					
Thalamus	211	0.003	-10 -16 6	L	4.45
Thalamus			-16 -24 8	L	4.18
Thalamus			-6 -24 8	L	372
Thalamus	125	0.038	12 -10 8	R	4.39
Medial Dorsal Nucleus: Heroin < Placebo Treatment					
Postcentral Gyrus	2194	< 0.001	-32 -30 52	L	4.99
Postcentral Gyrus			-50 -20 52	L	4.77
Precentral Gyrus			-12 -16 80	L	4.47
Middle Temporal Gyrus	764	< 0.001	58 -6 -24	R	4.89
Superior Temporal Gyrus			58 -4 -12	R	4.72
Middle Temporal Gyrus			58 -14 -16	R	4.67
Precentral Gyrus	1156	< 0.001	10 -20 80	R	4.64
Postcentral Gyrus			46 -26 58	R	4.60
Postcentral Gyrus			20 -36 54	R	4.40
Parahippocampal Gyrus	254	0.001	24 -16 -26	R	4.60
Parahippocampal Gyrus			28 -24 -16	R	4.44
Parahippocampal Gyrus			26 -34 -16	R	4.31
Precuneus	2045	< 0.001	10 -60 26	R	4.59
Precuneus			10 -54 34	R	4.52
Cerebellum			-24 -48 -22	L	4.51
Lateral Occipital Cortex	242	0.001	48 -68 26	R	4.38
Angular Gyrus			54 -58 34	R	3.22
Lateral Occipital Cortex	296	< 0.001	-46 -70 12	L	4.32
Angular Gyrus			-50 -50 22	L	4.17
Cerebellum			-52 -64 26	L	3.58
Parahippocampal Gyrus	149	0.017	-20 -14 -26	L	4.29
Parahippocampal Gyrus			-30 -26 -22	L	3.82
Temporal Fusiform Cortex			-30 -32 -28	L	3.64
Superior Frontal Gyrus	151	0.016	20 22 50	R	4.24
Middle Frontal Gyrus			30 34 46	R	3.20
Superior Frontal Gyrus	175	0.008	-58 -36 -4	L	4.18
Middle Frontal Gyrus			-52 -36 -10	L	4.11
Precentral Gyrus	183	0.006	-4 -20 54	L	4.07
Precentral Gyrus			0 -18 64	R	3.74
Precentral Gyrus			12 -16 58	R	3.57

Occipital Fusiform Gyrus	206	0.003	12 -90 -16	R	4.07
Lingual Gyrus			-4 -88 -8	L	3.90
Occipital Pole			4 -94 -4	R	3.74
Medial Dorsal Nucleus: Heroin > Placebo Treatment					
Thalamus	216	0.002	-14 -14 4	L	4.73
Thalamus			-10 -18 10	L	4.71
Thalamus			-2 -24 4	L	3.71
Thalamus	135	0.027	12 -18 12	R	4.33
Thalamus			10 -8 2	R	3.00

FWE: family-wise error; L: left; R: right.

Table 4: Resting-state associated regional homogeneity (ReHo)

Brain region	Cluster size (voxels)	Cluster p-value (FWE corrected)	MNI coordinats (x y z)	Hemisphere	Z-value at voxel level
Heroin > Placebo Treatment					
Posterior Cingulate Gyrus	639	< 0.001	2 -28 30	R	5.84
Posterior Cingulate Gyrus			-4 -44 12	L	3.30
Thalamus	1286	< 0.001	-10 -14 12	L	5.38
Thalamus			6 -8 4	R	5.33
Thalamus			-18 -34 12	L	4.83
Postcentral Gyrus	831	< 0.001	2 -38 62	R	4.66
Precentral Gyrus			0 -28 60	R	4.63
Precentral Gyrus			2 -20 70	R	4.50
Heroin < Placebo Treatment					
Temporal Pole	414	0.006	-50 2 -34	L	4.76
Inferior Temporal Gyrus			-56 -16 -26	L	4.17
Middle Temporal Gyrus			-58 -2 -26	L	4.01
Superior Frontal Gyrus	381	0.009	14 32 62	R	4.62
Middle Frontal Gyrus			38 22 58	R	4.29
Middle Frontal Gyrus			28 34 52	R	4.06
Lateral Occipital Cortex	401	0.007	-36 -90 22	L	4.25
Lateral Occipital Cortex			-36 -80 42	L	4.22
Lateral Occipital Cortex			-48 -78 24	L	4.04
Controls > Heroin Treatment					
Frontal Pole	259	0.039	20 56 30	R	6.12
Superior Frontal Gyrus			10 52 24	R	5.41
Frontal Pole			6 58 28	R	4.75
Middle Frontal Gyrus	299	0.021	-36 22 56	L	4.53
Middle Frontal Gyrus			-44 20 48	L	4.29
Superior Frontal Gyrus			-18 30 52	L	4.19
Controls < Heroin Treatment					
none					
Controls > Placebo Treatment					
none					
Controls < Placebo Treatment					
none					

FWE: family-wise error; L: left; R: right.

Table 5: Resting-state associated fractional amplitude of low frequency fluctuations (fALFF)

Brain region	Cluster size (voxels)	Cluster p-value (FWE corrected)	MNI coordinats (x y z)	Hemisphere	Z-value at voxel level
Heroin > Placebo Treatment					
Precentral Gyrus	540	0.001	2 -32 60	R	6.06
Precentral Gyrus			2 -22 68	R	5.62
Precentral Gyrus			-4 -26 54	L	3.93
Thalamus	554	< 0.001	-12 -20 8	L	4.71
Thalamus			-8 -12 8	L	4.67
Thalamus			8 -8 4	R	4.53
Heroin < Placebo Treatment					
none					
Controls > Heroin Treatment					
Posterior Cingulate Gyrus	563	0.001	-4 -50 32	L	5.13
Precuneus			12 -58 26	R	4.90
Precuneus			-10 -60 22	L	4.28
Controls < Heroin Treatment					
Cerebellum	273	0.039	-30 -58 -26	L	4.59
Cerebellum			-38 -58 -32	L	3.99
Cerebelum			-48 -70 -30	L	3.54
Midbrain	550	0.001	-10 -4 -10	L	4.37
Amygdala			-26 -2 -18	L	4.20
Midbrain			-14 -20 -14	L	3.81
Controls > Placebo Treatment					
none					
Controls < Placebo Treatment					
Cerebellum	310	0.024	28 -34 -32	R	3.93
Inferior Temporal Gyrus			46 -38 -24	R	3.69
Parahippocampal Gyrus			26 -20 -30	R	3.38

FWE: family-wise error; L: left; R: right.

Table 6: Differences in slow-5 and slow-4 amplitude of low frequency fluctuations (fALFF) bands in heroin and placebo treatment

Brain region	Cluster size (voxels)	Cluster p-value (FWE corrected)	MNI coordinats (x y z)	Hemisphere	Z-value at voxel level
fALFF slow-5: Heroin > Placebo Treatment					
Precentral Gyrus	1305	< 0.001	2 -20 70	R	4.29
Precentral Gyrus			20 -28 66	R	4.22
Precentral Gyrus			6 -28 70	R	4.13
fALFF slow-5: Heroin < Placebo Treatment					
none					
fALFF slow-4: Heroin > Placebo Treatment					
Thalamus	814	< 0.001	8 -8 4	R	4.96
Thalamus			-8 -12 8	L	4.97
Thalamus			8 -24 4	R	3.90
fALFF slow-4: Heroin < Placebo Treatment					
none					

fALFF slow-4: 0.027-0.073 Hz; fALFF slow-5: 0.01-0.027 Hz; FWE: family-wise error; L: left; R: right.

Table 7: Positive correlations of morphine metabolites and regional treatment difference values in thalamocortical functional connectivity (FC), regional homogeneity (ReHo) and fractional amplitude of low frequency fluctuations (fALFF).

Brain region	Cluster size (voxels)	Cluster p-value (FEW corrected)	MNI coordinates (x y z)	Hemisphere	Z-value at voxel level
Thalamocortical FC and morphine plasma levels					
Frontal Orbital Cortex	52	0.046	-24 12 -20	L	5.41
ReHo and morphine plasma levels					
none					
fALFF and morphine plasma levels					
none					

fALFF: fractional amplitude of low frequency fluctuations; FC: functional connectivity; FWE: family-wise error; L: left; R: right; ReHo: regional homogeneity.

Table 8: Negative correlation of first-order BIS attention score with fractional amplitude of low frequency fluctuations (fALFF).

Brain region	Cluster size (voxels)	Cluster p-value (FWE corrected)	MNI coordinates (x y z)	Hemisphere	Z-value at voxel level
fALFF (Heroin treatment) and first-order BIS					
Midbrain	238	0.049	4 -10 -14	R	4.20
Thalamus			10 -16 -6	R	4.17
Hypothalamus			8 -6 -8	R	3.34

BIS: Barrett Impulsiveness Scale; fALFF: fractional amplitude of low frequency fluctuations; FWE: family-wise error; L: left; R: right; ReHo: regional homogeneity.

FIGURES

Figure 1: Thalamocortical FC differences between heroin and placebo treatment in heroin-dependent patients. Red indicates increased FC by heroin and blue indicates reduced FC relative to the placebo treatment.

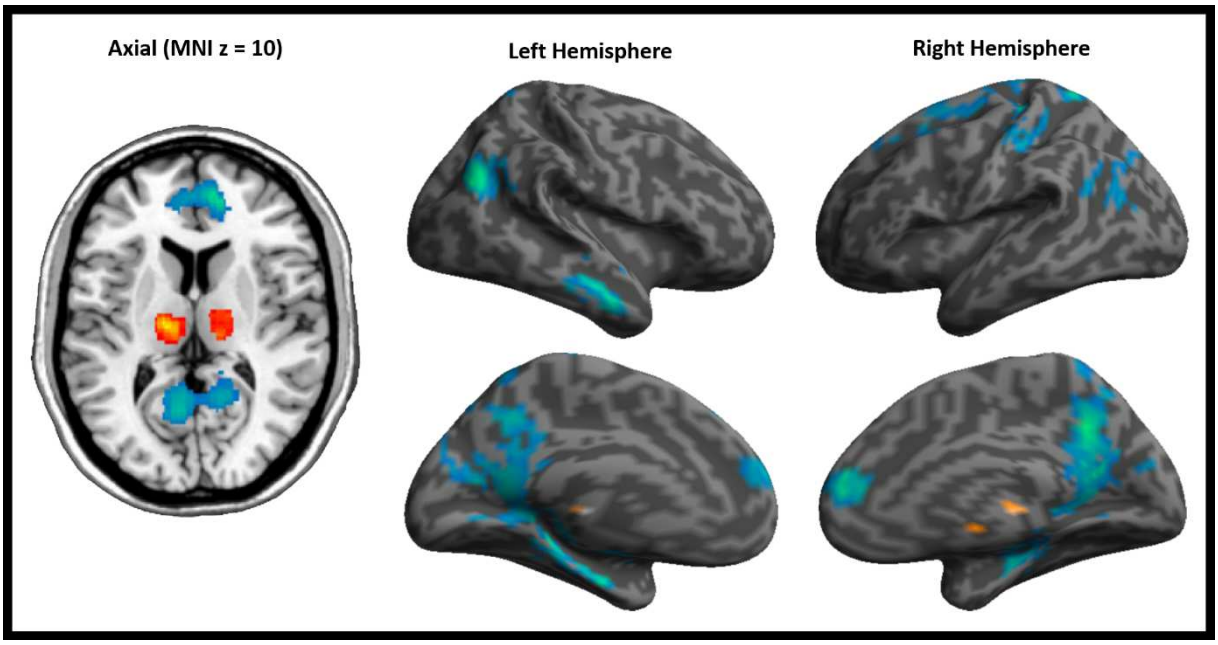

Figure 2: Significant increase in ReHo and fALFF values induced by acute heroin compared with placebo treatment. Spectral colours indicate mean thalamic ReHo and fALFF values of heroin and placebo treatment.

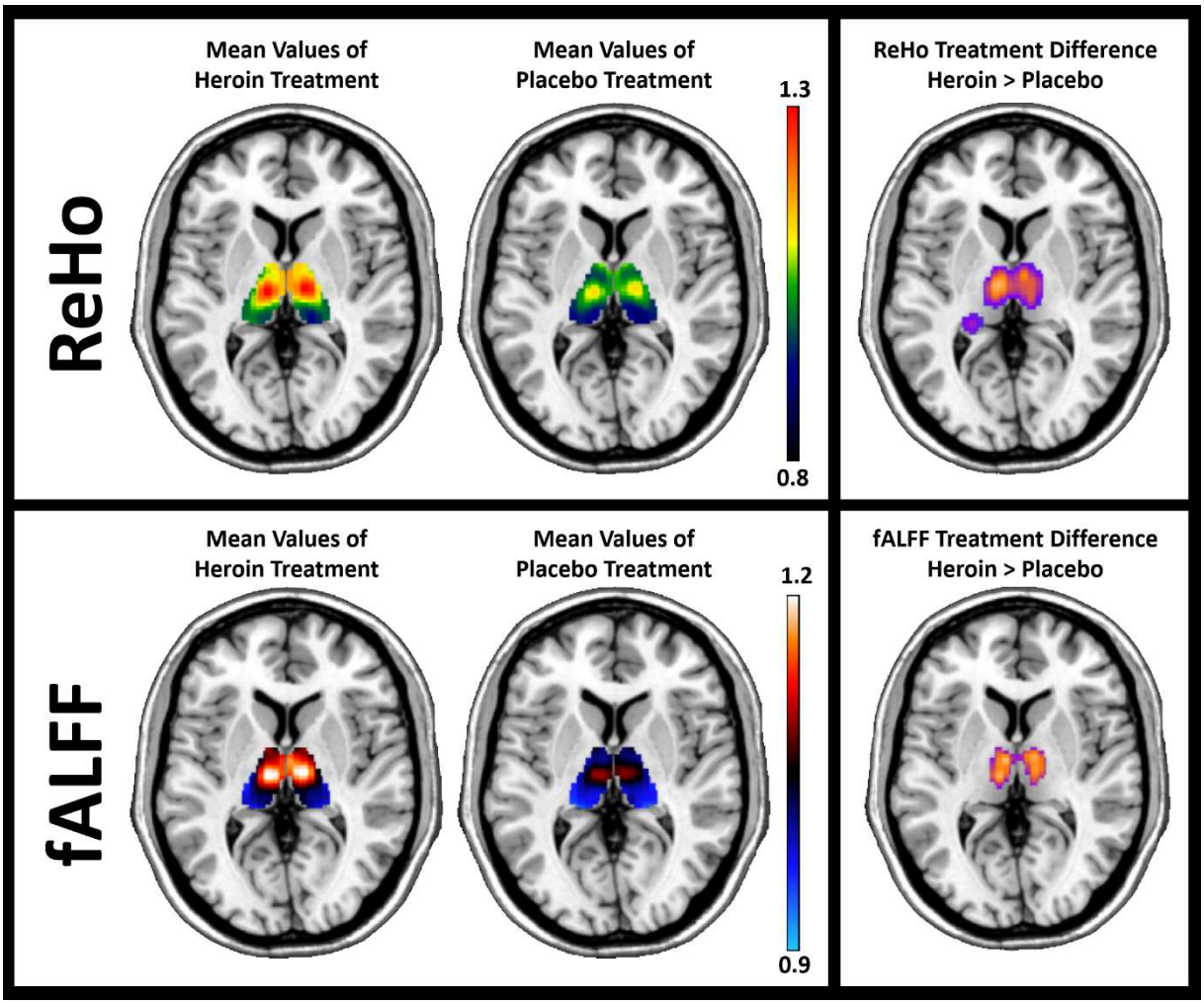

Figure 3: Negative correlation of first-order BIS attention score with fractional amplitude of low frequency fluctuations (fALFF). The correlation plot is shown only for visualization purpose and doesn't represent statistical inference.