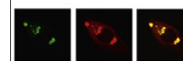


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Research Report

Craving correlates with mesolimbic responses to heroin-related cues in short-term abstinence from heroin: An event-related fMRI study

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ABSTRACT

Craving is an important factor in relapse to drug abuse, and cue-induced craving is an especially powerful form of this construct. Neuroimaging methods have been utilized to study drug cue-induced craving and neural correlates in the human brain. However, very few studies have focused on characterizing craving and the neural responses to heroin-related cues in short-term abstinent heroin-dependent patients. Twenty-four heroin-dependent subjects and 20 demographically matched drug-naïve subjects participated in this study. An event-related cue-reactivity paradigm was employed, while changes in blood oxygen level-dependent (BOLD) signals were acquired by functional magnetic resonance imaging (fMRI). The heroin-dependent group reported significantly increased craving following exposure to heroin-related cues. Direct comparison between the two groups showed that brain activation to heroin-related minus neutral cues was significantly greater for the heroin-dependent group in the bilateral nucleus accumbens (NAc), caudate, putamen, amygdala, hippocampus/parahippocampus, midcingulate cortex, dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), medial frontal gyrus (MeFG), mid-brain, thalamus, left anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), and

Abbreviations: BOLD, blood oxygen level-dependent; fMRI, functional magnetic resonance imaging; NAc, nucleus accumbens; DLPFC, dorsolateral prefrontal cortex; OFC, orbitofrontal cortex; MeFG, medial frontal gyrus; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; DA, dopamine; PFC, prefrontal cortex; VTA, ventral tegmental area; DLPFC, dorsolateral prefrontal cortex; SFG, superior frontal gyrus; PoG, postcentral gyrus; PrG, precentral gyrus; MCC, midbrain, midcingulate cortex; MOG, middle occipital gyrus; STG, superior temporal gyrus; MTG, middle temporal gyrus; ITG, inferior temporal gyrus; IPL, inferior parietal lobule; SPL, superior parietal lobule; IOG, inferior occipital gyrus; MFG, middle frontal gyrus; Hp/ParaHp, hippocampus/parahippocampus

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subcallosal gyrus. Changes in craving in the heroin-dependent group correlated positively with brain activation in the bilateral NAc, caudate, right putamen, and left ACC. The abstinence duration correlated positively with brain activation in the left caudate and right parahippocampal gyrus. In conclusion, the cue-reactivity paradigm significantly activated neural responses in the mesolimbic dopamine (DA) system and prefrontal cortex (PFC) and induced increased craving in short-term abstinent heroin-dependent patients. We suggest that these response patterns characterize the high vulnerability of relapse in short-term abstinent heroin-dependent subjects.

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1. Introduction

Drug addiction is a major health problem in modern society (Nordahl et al., 2005). Heroin addiction in particular has become an increasingly serious problem for China in recent years (Tang et al., 2006; Xiao et al., 2006). Such addiction is characterized by the failure to resist one's impulses to obtain and take drugs despite serious and negative consequences (Volkow and Li, 2004). It is assumed that the molecular and structural changes mainly within the mesolimbic dopamine (DA) system constitute the 'switch' from controlled drug intake to compulsive drug abuse (Spanagel and Heilig, 2005). Heroin craving is a trigger for relapse and dropping out of treatment (Fareed et al., 2010). It may be induced in addicted patients using drug-related cues known to prompt drug-seeking behavior (Zijlstra et al., 2009). As is shown, short-term abstinence is very difficult for heroin-dependent patients (Shi et al., 2007) and relapse was highly associated with shorter treatment duration for drug addicted individuals (Xie et al., 2011). The neural mechanism underlying high vulnerability to relapse during short-term abstinence is not very clear. Investigation of heroin cues-induced craving and brain response may be a key to solving the problem.

Neuroimaging studies on craving have mostly investigated cocaine (Kufahl et al., 2005; Sinha et al., 2005; Wexler et al., 2001; Wong et al., 2006). However, there are very few imaging studies focusing on heroin craving in short-term abstinent heroin-dependent individuals or which explore the role of drug-cue-induced craving in this regard. Some studies using cue-reactivity paradigms have been conducted on opioid-dependent but not heroin-dependent subjects. These studies have indicated that the mesolimbic DA system is mainly involved in the heroin-cues induced reactivity such as the nucleus accumbens (NAc), caudate, putamen (Wang et al., 2011a), anterior cingulate cortex (ACC) (Daglish et al., 2001; Langleben et al., 2008; Wang et al., 2011a), amygdala (Mei et al., 2010), hippocampus (Langleben et al., 2008; Mei et al., 2010), and ventral tegmental area (VTA) (Langleben et al., 2002; Mei et al., 2010; Zijlstra et al., 2009). Also, the prefrontal areas such as the medial prefrontal cortex (Daglish et al., 2001), orbitofrontal cortex (OFC) (Langleben et al., 2008; Mei et al., 2010), dorsolateral prefrontal cortex (DLPFC) (Wang et al., 2011a) and other brain regions including the thalamus (Langleben et al., 2002; Mei et al., 2010; Wang et al., 2011a), posterior cingulate cortex (PCC) (Langleben et al., 2002; Wang et al., 2011a), insula (Langleben et al., 2002; Langleben et al., 2008), subthalamic nucleus (Zijlstra et al., 2009) and midbrain (Sell et al., 1999) are involved in heroin-cues induced

reactivity. However, few of the studies mentioned above showed a definite association between cue-elicited craving and brain activation in response to heroin-related cues in opioid-dependent patients. The results derived from studies with opioid-dependent patients cannot be directly linked to heroin addiction because of the potential impact of other opioids such as methadone (Langleben et al., 2008) and buprenorphine (Mei et al., 2010). Meanwhile, the potential relationship between craving and brain activation in response to heroin cues in short-term abstinent heroin-dependent patients is currently unknown.

Neuroimaging studies in drug addiction have demonstrated that the mesolimbic DA system is affected structurally (Wang et al., 2011b), metabolically (Schweitzer et al., 2011), functionally (Kufahl et al., 2005). The mesolimbic DA system has been suggested to be mainly involved in the mediation of cue-induced heroin craving (Wise, 2009). Two cocaine studies by Volkow and associates have demonstrated that cocaine cues significantly increase DA in the dorsal striatum mainly involved in the mesolimbic DA system, and the magnitude of this increase is correlated with subjective craving (Volkow et al., 2006; Wong et al., 2006). Based on the previous finding of studies on opioid-dependent patients, we hypothesized that heroin-related cues would significantly activate the brain regions mainly involved in the mesolimbic DA system and the prefrontal cortex (PFC) compared with neutral cues. We further predicted that the increase in drug-cue-induced craving would be positively correlated with an increase in the activation of the mesolimbic DA system.

Moreover, the cue stimuli in most previous functional imaging studies of heroin addiction were shown in a block design (Yang et al., 2009; Zijlstra et al., 2009). They analyzed brain activation during the block for a relatively long time (e.g. 18–30 s) under repeated heroin-related cue exposure. The paradigms could not indicate the 'immediate response' to drug cues (Ko et al., 2011). An event-related design may facilitate a characterization of the temporal response profiles that has a high effective temporal resolution relative to the repetition time (Friston et al., 1998). Even an event-related analysis can provide a more accurate model of the hemodynamic responses than an epoch-related analysis in a block design (Mechelli et al., 2003). Therefore, event-related fMRI studies are needed to determine the immediate reaction to heroin-related cues and to characterize the nature of the cue-induced craving response.

In the present study, we utilized an event-related fMRI cue-reactivity paradigm to test this hypothesis in short-term abstinent heroin-dependent patients.

2. Results

2.1. Craving ratings

The subjective craving score before cue presentation was 2.5 ± 1.7 (mean \pm SD) and after cue presentation was 3.4 ± 2.6 (mean \pm SD). A paired sample t-test indicated that subjective craving scores after cue presentation were significantly higher than before cue presentation for the heroin-dependent patients ($t = 2.82$, $P = 0.01$).

2.2. Imaging results

2.2.1. The within-group differences

The activated brain regions relating to the “Heroin-Neutral” contrast in the heroin-dependent group included the bilateral NAc, caudate, putamen, ACC, DLPFC, OFC, superior frontal gyrus (SFG), postcentral gyrus (PoG), precentral gyrus (PrG), amygdala, pons, midbrain, midcingulate cortex (MCC), fusiform, PCC, precuneus, thalamus, middle occipital gyrus (MOG), superior temporal gyrus (STG), middle temporal gyrus (MTG), inferior temporal gyrus (ITG), inferior parietal lobule (IPL), superior parietal lobule (SPL), cerebellum, left hippocampus, insula, inferior occipital gyrus (IOG) and right middle frontal gyrus (MFG) (Table 2 and Fig. 1). No supra-threshold activation in relation to the heroin-related cues was observed in the control group.

2.2.2. The between-group differences

The two-sample t-test indicated that the heroin group, compared to the control group, had significantly higher activation in the following regions: bilateral NAc, caudate, putamen, DLPFC, OFC, MeFG, amygdala, hippocampus/parahippocampus (Hp/ParaHp), MCC, midbrain, thalamus, left ACC, PCC, and subcallosal gyrus. No significant brain activation was found for the control group compared to the heroin-dependent group in response to the heroin-related cues (Table 3 and Fig. 2).

2.3. Correlation results

Significant positive correlations between changes in craving and brain responses to heroin-related cues were found for the bilateral NAc, caudate, right putamen, and left ACC. Significant positive correlations between abstinence duration and responses to heroin-related cues were found for the left caudate and right parahippocampal gyrus. These results were still significant even after covarying for cigarettes per day (Fig. 3).

3. Discussion

Our event-related study revealed that heroin-related cues elicited increased activation in the mesolimbic DA and PFC regions of the brain in short-term abstinent heroin-dependent patients. Furthermore, the activation in the mesolimbic DA regions was associated with the craving for heroin. These findings suggest that the mesolimbic DA and PFC regions were associated with the mechanism of heroin craving under cue exposure.

Our results support the model suggested by Volkow proposing a network of interacting circuits underlying addiction (Volkow et al., 2011b). In our study, enhanced activity in the NAc, caudate and VTA in response to heroin-related cues indicated increased reward-based cognitive processes in the presence of the cues (Bush et al., 2002; Heinz et al., 2009). Enhanced activity in the amygdala and Hp/ParaHp reflects increased emotional and memorial processing of sensory stimuli (McClernon et al., 2005; Phan et al., 2002; Robbins et al., 2008). Enhanced activity in the OFC, MeFG and putamen is in line with increased motivation processes (Kufahl et al., 2005; Volkow et al., 2011b; Yalachkov et al., 2009), and finally, enhanced activity in the ACC and DLPFC reflects abnormal executive control process (Wang et al., 2011a). Interpretations of enhanced response in these areas have been suggested in the literature. One interpretation is that during addiction, the saliency value of a drug and its related cues are enhanced whilst inhibitory control is weakened, setting up a positive-feedback loop which is perpetuated by the enhanced activation of the motivation/drive and memory circuits. As a

Table 1 – Demographic and clinical characteristics of participants.

| Characteristics | Heroin-dependent N = 24 | Control N = 20 | t-Value | P-value |
|---------------------------------|----------------------------|-------------------|---------|---------|
| Age | 32.8 \pm 6.6 | 35.0 \pm 7.0 | −1.14 | 0.26 |
| Range | 23–44 | 19–46 | – | – |
| Years of education | 10.9 \pm 3.1 | 10.1 \pm 2.3 | 0.98 | 0.33 |
| Range | 6–17 | 6–14 | – | – |
| Cigarettes (per day) | 17.8 \pm 5.9 | 15.0 \pm 5.0 | 1.82 | 0.08 |
| Range | 5–30 | 10–20 | – | – |
| Duration of heroin use (months) | 78.6 \pm 50.1 | – | – | – |
| Range | 12–229 | – | – | – |
| Average heroin dose (g/day) | 1.0 \pm 1.2 | – | – | – |
| Range | 0.1–5 | – | – | – |
| Total heroin dose (g) | 2171.1 \pm 2484.9 | – | – | – |
| Range | 108–8400 | – | – | – |
| Abstinence (days) | 21.7 \pm 16.0 | – | – | – |
| Range | 7–72 | – | – | – |

Table 2 – Activated areas for the heroin-dependent group in response to heroin-related vs. neutral cues.

| Brain regions | Brodmann's area | Peak location | | | Peak t-score | Voxel number |
|---------------|------------------|---------------|-----|-----|--------------|--------------|
| | | x | y | z | | |
| ACC | | | | | | |
| L | 24, 25 | −3 | 15 | −6 | 6.49 | 43 |
| R | 24, 25, 33 | 3 | 18 | −6 | 5.29 | 38 |
| Amygdala | | | | | | |
| L | – | −21 | 0 | −21 | 5.12 | 34 |
| R | – | 21 | −3 | −21 | 6.81 | 36 |
| Caudate | | | | | | |
| L | – | −9 | 6 | 12 | 4.67 | 44 |
| R | – | 12 | 9 | 12 | 5.84 | 70 |
| Cerebellum | | | | | | |
| L | – | −12 | −72 | −42 | 4.52 | 30 |
| R | – | 18 | −75 | −45 | 4.00 | 13 |
| DLPFC | | | | | | |
| IFG | | | | | | |
| L | 6, 9, 45, 46, 47 | −54 | 9 | 33 | 5.64 | 86 |
| R | 6, 9, 46, 47 | 48 | 9 | 30 | 5.27 | 63 |
| MFG | | | | | | |
| R | 6, 8, 9, 11, 46 | 36 | −9 | 48 | 5.50 | 128 |
| MeFG | | | | | | |
| L | 8, 9, 10 | −3 | 63 | −3 | 4.32 | 41 |
| R | 6, 8, 9 | 4 | 48 | 36 | 4.51 | 48 |
| Fusiform | | | | | | |
| L | 37 | −45 | −45 | −24 | 5.99 | 64 |
| R | 37 | 48 | −45 | −24 | 5.13 | 35 |
| Hippocampus | | | | | | |
| L | – | −30 | −12 | −18 | 4.07 | 10 |
| Insula | | | | | | |
| L | – | −39 | −6 | 12 | 3.44 | 10 |
| IOG | | | | | | |
| L | 18, 19 | −30 | −96 | −12 | 4.61 | 17 |
| IPL | | | | | | |
| L | 7, 40 | −57 | −30 | 36 | 8.23 | 221 |
| R | 7, 40 | 36 | −54 | 57 | 4.22 | 42 |
| ITG | | | | | | |
| L | 19, 20, 37 | −57 | −69 | −6 | 8.08 | 39 |
| R | 19, 37 | 54 | −69 | −3 | 7.34 | 25 |
| MCC | | | | | | |
| L | 23, 24, 31 | 0 | −36 | 33 | 7.37 | 98 |
| R | 23, 24, 31 | 3 | 0 | 36 | 7.29 | 75 |
| Midbrain | | | | | | |
| L | – | −3 | −30 | −6 | 7.74 | 182 |
| R | – | 9 | −27 | −6 | 6.81 | 116 |
| MOG | | | | | | |
| L | 18, 19, 37 | −57 | −66 | −9 | 8.20 | 58 |
| R | 18, 19, 37 | 54 | −66 | −12 | 6.73 | 37 |
| MTG | | | | | | |
| L | 19, 37, 39 | −54 | −63 | −6 | 7.66 | 51 |
| R | 19, 21, 37, 39 | 51 | −66 | 0 | 6.98 | 82 |
| NAc | | | | | | |
| L | – | −6 | 15 | −6 | 6.04 | 20 |
| R | – | 7 | 12 | −7 | 5.00 | 17 |
| OFC | | | | | | |
| MFG | | | | | | |
| L | 6, 9, 11, 46 | −30 | 39 | −12 | 5.20 | 43 |
| R | 11, 47 | 28 | 37 | −11 | 5.22 | 27 |
| PCC | | | | | | |
| L | 23, 29, 30, 31 | −3 | −54 | 21 | 7.42 | 64 |
| R | 23, 29, 30, 31 | 3 | −60 | 21 | 6.45 | 52 |
| PoG | | | | | | |
| L | 2, 5, 40 | −57 | −27 | 39 | 6.83 | 42 |
| R | 1, 2, 3 | 66 | −24 | 33 | 4.80 | 30 |

Table 2 (continued)

| Brain regions | Brodmann's area | Peak location | | | Peak t-score | Voxel number |
|---------------|-----------------|---------------|-----|-----|--------------|--------------|
| | | x | y | z | | |
| Pons | | | | | | |
| L | – | –6 | –21 | –24 | 5.03 | 29 |
| R | – | 15 | –18 | –24 | 5.34 | 52 |
| Precuneus | | | | | | |
| L | 7, 19, 31 | –24 | –81 | 45 | 5.44 | 113 |
| R | 7 | 3 | –63 | 18 | 4.84 | 18 |
| PrG | | | | | | |
| L | 6 | –48 | 0 | 33 | 5.17 | 33 |
| R | 6 | 42 | –3 | 45 | 6.08 | 32 |
| Putamen | | | | | | |
| L | – | –12 | 9 | –6 | 3.58 | 10 |
| R | – | 15 | 9 | 3 | 4.57 | 12 |
| SFG | | | | | | |
| L | 8, 9 | –6 | 45 | 51 | 3.73 | 25 |
| R | 8, 9 | 3 | 51 | 39 | 4.82 | 42 |
| SPL | | | | | | |
| L | 7 | –33 | –75 | 45 | 6.40 | 93 |
| R | 7 | 33 | –66 | 54 | 5.33 | 66 |
| STG | | | | | | |
| L | 38 | –33 | 6 | –21 | 4.68 | 11 |
| R | 22, 39 | 42 | –60 | 15 | 4.55 | 23 |
| Thalamus | | | | | | |
| L | – | –3 | –12 | 0 | 5.47 | 59 |
| R | – | 3 | –12 | 3 | 5.85 | 68 |

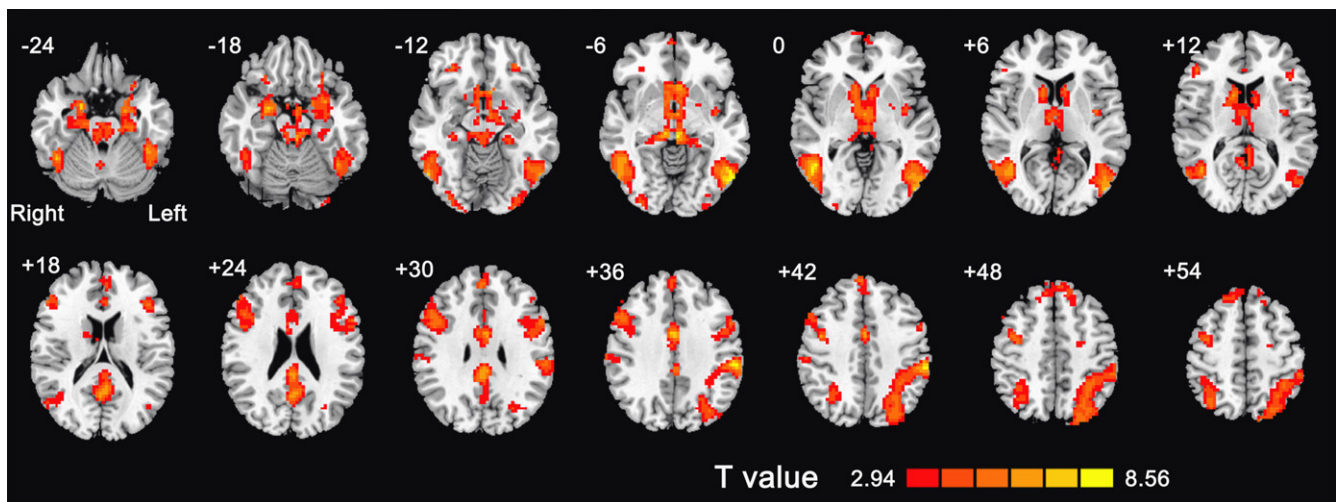


Fig. 1 – The activated regions relating to the “Heroin-Neutral” contrast for the heroin-dependent group ($P < 0.05$, corrected for FDR, a minimum of 10 voxels).

result, this could lead to compulsive drug seeking without regard for the resultant negative consequences.

Most importantly, our findings demonstrated that greater activation in mesolimbic DA areas including the bilateral NAc, caudate, right putamen and left ACC was associated with greater craving for heroin use. All of these regions are involved in the reward pathway and underlie motivated behavior and the attribution of incentive salience (Filbey et al., 2009; McClernon et al., 2009; Mei et al., 2010; Wang et al., 2007; Zijlstra et al., 2009). Meanwhile, increased activation of these areas during cue-elicited craving paradigms has

also been associated with a greater likelihood of subsequent relapse after treatment in alcohol- and cocaine-dependent patients (Grusser et al., 2004; Sinha et al., 2005). Therefore, we postulate that the abnormal association between craving and activation of these regions could be responsible for the high susceptibility of relapse in heroin addiction, especially during short-term abstinence.

We also found significant positive correlations between the duration of abstinence and responses to heroin-related cues for both the left caudate and right parahippocampal gyrus. The parahippocampal gyrus plays a role in visuo-spatial

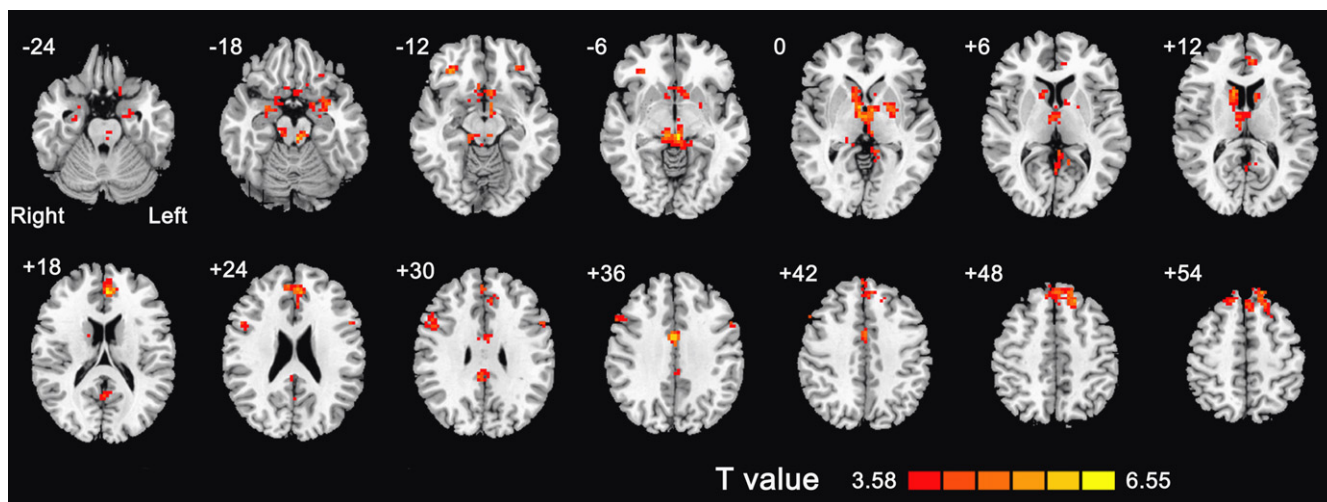


Fig. 2 – The differences relating to the “Heroin-Neutral” contrast between the heroin-dependent and control groups ($P < 0.05$, corrected for FDR, a minimum of 10 voxels).

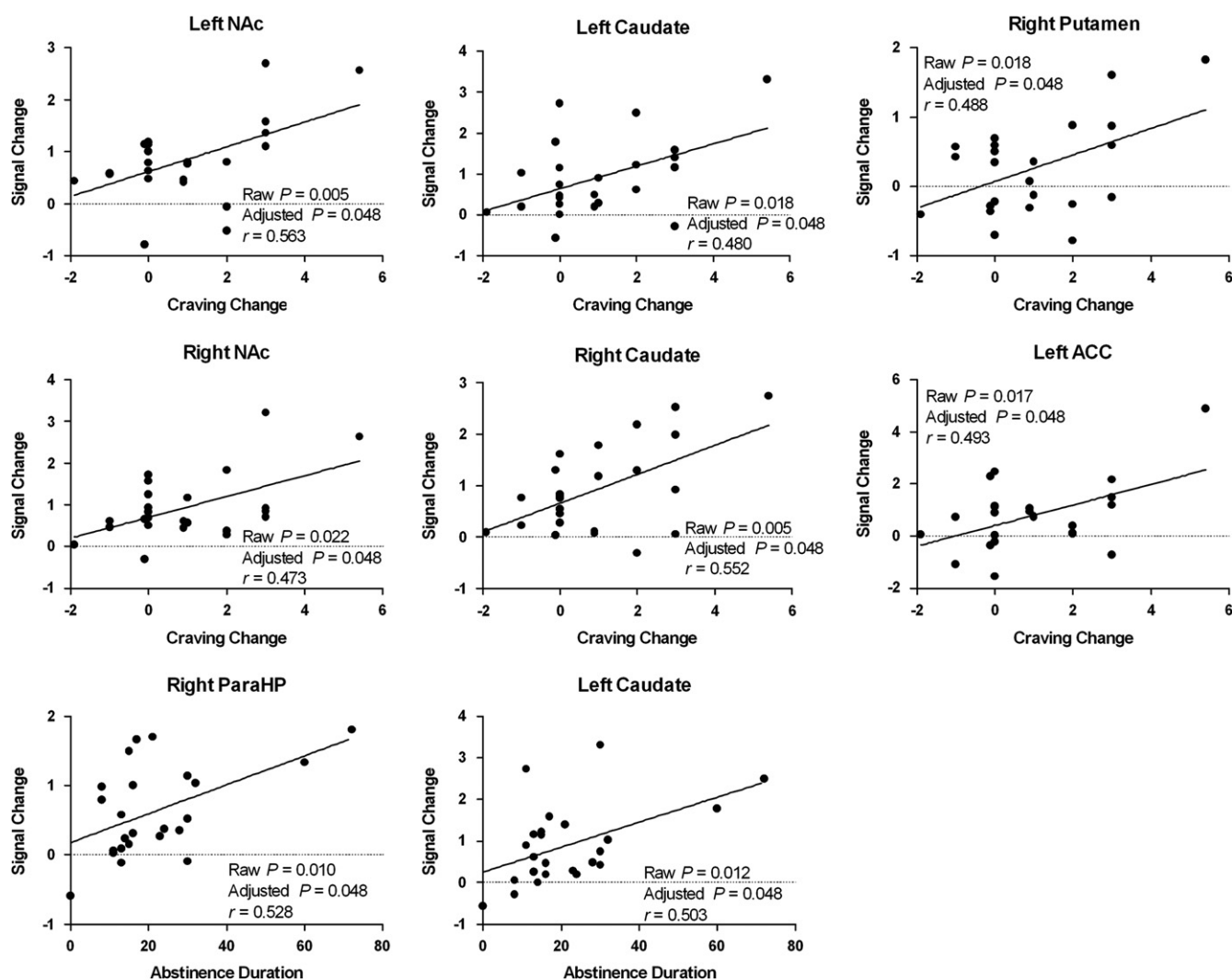


Fig. 3 – Correlation maps between the signal amplitude in the ROI, which were significantly activated among the heroin group by heroin-related cues, and craving changes, abstinence duration (r : correlation coefficient, P : P value).

Table 3 – Activated brain areas for the heroin-dependent group in contrast to the control group in response to heroin-related vs. neutral cues.

| Brain regions | Brodmann's area | Peak location | | | Peak t-score | Voxel number |
|---------------|---------------------|---------------|-----|-----|--------------|--------------|
| | | x | y | z | | |
| ACC | | | | | | |
| L | 24, 25, 32 | −6 | 42 | 15 | 4.89 | 40 |
| Amygdala | | | | | | |
| L | – | −24 | −6 | −21 | 4.30 | 18 |
| R | – | 24 | 0 | −21 | 5.01 | 18 |
| Caudate | | | | | | |
| L | – | −9 | 6 | 12 | 4.39 | 15 |
| R | – | 12 | 12 | 12 | 5.49 | 62 |
| DLPFC | | | | | | |
| IFG | | | | | | |
| L | 9, 45, 47 | −57 | 12 | 33 | 4.55 | 31 |
| SFG | | | | | | |
| L | 6, 8, 9 | −18 | 30 | 54 | 5.02 | 53 |
| R | 8, 9 | 9 | 42 | 51 | 4.68 | 23 |
| Hp/ParaHp | | | | | | |
| L | 28, 30, 34, 35, 36 | −9 | −45 | 3 | 4.68 | 34 |
| R | 27, 30, 34, 35, 36 | 27 | −15 | −30 | 4.43 | 18 |
| MCC | | | | | | |
| L | 23, 24, 31, 32 | −3 | −3 | 33 | 5.28 | 32 |
| R | 23, 24, 31 | 3 | 0 | 39 | 6.55 | 30 |
| MeFG | | | | | | |
| L | 6, 8, 9, 10, 11, 25 | −3 | 45 | 18 | 5.76 | 60 |
| R | 6, 8, 9, 11, 25 | 6 | 48 | 24 | 4.84 | 44 |
| Midbrain | | | | | | |
| R | – | 12 | −27 | −15 | 5.71 | 69 |
| R | – | −3 | −30 | −6 | 6.17 | 89 |
| NAc | | | | | | |
| L | – | −9 | 12 | −6 | 4.78 | 17 |
| R | – | 8 | 13 | −6 | 4.05 | 10 |
| OFC | | | | | | |
| IFG | | | | | | |
| R | 47 | 33 | 33 | −12 | 5.14 | 10 |
| MFG | | | | | | |
| L | 6, 9, 11, 46, 47 | −30 | 39 | −12 | 4.15 | 14 |
| R | 6, 9, 11, 46, 47 | 33 | 36 | −12 | 5.45 | 27 |
| PCC | | | | | | |
| L | 23, 29, 30, 31 | −12 | −54 | 6 | 4.35 | 18 |
| Putamen | | | | | | |
| L | – | −21 | 0 | 0 | 4.80 | 44 |
| R | – | 24 | 3 | 0 | 3.68 | 11 |
| SuG | | | | | | |
| L | 25, 34 | −6 | 12 | −12 | 4.95 | 12 |
| Thalamus | | | | | | |
| L | – | −3 | −9 | 3 | 5.23 | 34 |
| R | – | 3 | −12 | 3 | 5.74 | 55 |

attention (Janse Van Rensburg et al., 2009). The activation of the parahippocampal gyrus possibly suggests enhanced attention to the heroin-related cues. The correlations mentioned above may indicate that in the period of short-term abstinence, the craving for heroin and subsequent possibility of relapse would increase with the abstinence duration in abstinent heroin subjects. A study of animal models for drug addiction also demonstrated that the longer the abstinence duration, the greater the chance of relapse (Schumann et al., 2008). Our results are consistent with the observations in this animal study. Incubation of craving has also been observed in rats with a history of some drugs of abuse such as

methamphetamine (Mann et al., 2008), alcohol (Hampp et al., 2008), and nicotine (Vengeliene et al., 2008). These results provide some evidence of incubation in the heroin-dependent subjects.

Besides the heroin dependence, prescription opioid abuse and addiction are rapidly growing in number. Numerous challenges are met in finding appropriate therapies (Holmes, 2012). Our findings suggest that future therapies for either heroin dependence or prescription opioid abuse and addiction should assess cue-induced brain responses prior to treatment as an indicator of relapse potential. Furthermore, changes in drug-cue-induced brain responses

after treatment may be a reliable marker of treatment efficacy. Therapies for heroin addiction, prescription opioid abuse and other drug addiction which would block such responses to heroin-related cues would presumably reduce susceptibility for continued drug abuse.

The current study has several potential limitations. First, this is a study on male heroin-dependent users. A recent study by Volkow et al. (2011a) indicated that female cocaine addicts demonstrated different brain metabolism compared to male addicts following drug-cue exposure. Additionally, due to the difficulty of data collection and the low availability of female patients, we had to restrict the experimental sample to males. Therefore, it is currently unclear whether our findings can be applied to heroin-dependent females. Second, we used a conscious (explicit) self-report measure of craving as the dependent variable. Our study did not enable us to assess whether the two groups differed in unconscious (implicit) responses to heroin-related cues. Third, since all of the heroin-dependent subjects were smokers and smoking was not the major focus of this study, we made a statistical match between the two groups. We believe the effect of nicotine was counterbalanced when conducting the between-group analysis and was regressed out when conducting the within-group correlation analysis for the heroin group.

In conclusion, this study demonstrated that in short-term abstinent heroin-dependent patients, heroin-related cues induced greater activation of most brain regions involved in the mesolimbic DA system and PFC. Increase in craving was positively associated with changes in the activation of the mesolimbic DA brain regions. These findings suggest that short-term abstinent heroin-addicted patients remained highly responsive to salient drug cues and therefore had a high vulnerability for relapse. The present study contributed to the scant but much-needed functional brain imaging literature on heroin addiction in the ongoing struggle against this disorder.

4. Experimental procedures

4.1. Participants

Twenty-four short-term abstinent heroin-dependent patients were recruited from residential treatment programs in Tangdu Hospital. Twenty healthy control subjects were recruited through advertisements (Table 1). All of the subjects were male smokers. Subjects completed a clinical interview prior to inclusion in the study (Structured Clinical Interview for DSM-IV [SCID], a urine drug screening, naloxone test and an fMRI task). Inclusion criteria for the heroin-dependent group were (1) DSM-IV criteria for heroin dependence for at least 1 year without use of any other opioid such as methadone and buprenorphine; (2) aged 18–50 years old; (3) right-handed; and (4) completion of detoxification treatment with no somatic symptoms of withdrawal and negative morphine urinalysis and naloxone tests. Exclusion criteria for all of the subjects were: (1) current or past psychiatric illness other than heroin and nicotine dependence; (2) neurological signs and/or history of neurological disease; (3) history of head

trauma; (4) history of cardiovascular or endocrine disease; (5) current medical illness or recent medicine use; (6) presence of magnetically active objects in the body; and (7) claustrophobia or any other medical condition that would preclude the patient from lying in the MRI scanner for approximately 40 min. All aspects of the research protocol were reviewed and approved by the ethics committee of Tangdu Hospital. All subjects gave written informed consent.

4.2. Design and procedure

We utilized an established event-related paradigm in this study (Wang et al., 2011a). There were 48 trials in total, consisting of 24 heroin-related cues and 24 neutral cues. The heroin-related cues were taken from our previous study (Wang et al., 2011a) and included images of heroin injection, preparation, and paraphernalia. The neutral cues included images of household objects or chores. All cues were projected onto a mirror system mounted on the scanner head coil, and presented in a pseudo-randomized order. Image cues were presented for 2 s with a variable 4–12-s inter-stimulus interval (mean = 8 s) during which a crosshair was displayed. The task began with a 10 s dummy scan followed by experimental scanning and the first cue (heroin-related or neutral). The total task time was 490 s. Cue presentations were delivered using the E-Prime software package (Psychology Software Tools, Inc., Pittsburgh). The timing of the cue presentation was synchronized with trigger pulses from the MRI scanner to ensure precise temporal integration of stimulus presentation and fMRI data acquisition. Participants were placed in the scanner in a supine position using a foam head holder to reduce motion. Earplugs were used to lessen scanner noise. No use of cigarettes, alcohol, tea, caffeine and any other drug or medicine was allowed 4 h prior to the time of the MRI scan.

For the heroin-dependent group, subjective heroin craving was assessed on a 0–10 visual analog scale (VAS) (Wang et al., 2011a) using the question, “To what extent do you feel the urge to use heroin?” Craving ratings (0 for the least craving and 10 for the strongest craving) were obtained before and immediately after each MRI scan, that is, pre- and post-cue presentation.

4.3. MRI data acquisition

All imaging data were acquired on a 3T MRI scanner (GE Signa Excite HD). Prior to formal experimental scanning, subjects underwent ‘mock scans’ for 1 min in order to become familiar with the scanning environment. Following the mock scanning session, 32 axial slices covering the whole brain were acquired with a T2*-weighted gradient-echo echo planar imaging pulse sequence (GE-EPI, TR = 2000 ms, TE = 30 ms, flip angle = 90°, matrix = 64 × 64, FOV = 256 × 256 mm², slice thickness = 4 mm, gap = 0 mm; spatial resolution = 4 × 4 × 4 mm³) for BOLD functional imaging. The corresponding high-resolution fast spoiled gradient echo 3D T1-weighted images were also collected for use as anatomical overlays of the functional data and for spatial normalization of the data sets to a standard atlas (TR = 7.8 ms, TE = 3.0 ms,

matrix = 256×256 , FOV = $256 \times 256 \text{ mm}^2$, spatial resolution = $1 \times 1 \times 1 \text{ mm}^3$). The structural data were checked by an experienced radiologist to assure that there were no structural abnormalities.

4.4. Data analysis

Functional image analysis was carried out with SPM8 software (<http://www.fil.ion.ucl.ac.uk/spm>). Images were slice-time corrected, motion corrected, normalized to the SPM EPI template, interpolated to 3 mm isotropic voxels, and spatially smoothed (Gaussian filter of the 8 mm kernel). Participants with excessive head motion (more than 1.5 mm in translation or 1° in rotation) were excluded from the analysis. The time-series fMRI data were filtered using a high pass filter and cut-off at 128 s. A statistical model for each subject was computed by applying a canonical response function (Friston et al., 1998). Regionally specific condition effects were tested by employing linear contrasts for each subject and different conditions. The critical contrast of interest was the heroin-related vs. neutral cues contrast (“Heroin-Neutral” contrast) that would reveal brain activations related to processing of heroin-related cues (Langleben et al., 2008). We directly compared the two groups of subjects (heroin-dependent vs. healthy controls) using voxel-wise random effects two-sample t-tests to identify regions in which brain activation to heroin-related cues differed between the two groups. Statistical significance was set at $P < 0.05$ (false discovery rate [FDR] corrected). Cluster size threshold was set at a minimum of 10 voxels in all whole brain analyses. All coordinates reported were in the Montreal Neurological Institute space.

In a second step, these regions involved in the mesolimbic DA system were then subject to a post hoc region of interest (ROI) analysis to identify the specific pattern of brain responses to the heroin-related cues in the heroin-dependent group. We chose the peak coordinate voxels of every anatomical region as centers of the sphere-shaped ROIs (radius = 3 mm). With the exclusion of the voxels of white matter, the remaining voxels in the ROIs were used for the correlation analysis. We extracted the raw data of heroin-dependent individuals from those significant brain regions that were observed in the comparison analysis between the two groups. Pearson correlations were conducted between changes of magnitude of brain activation in response to heroin-related cues and patients’ heroin use history (the total amount of heroin used, duration of heroin use, and duration of abstinence). To evaluate the association between changes in craving and brain responses to heroin-related cues, difference scores were calculated (post-cue presentation minus pre-cue presentation) for the behavioral measure of self-reported craving, and for the brain measures of BOLD activation to heroin-related cues. Analyses were performed using SPSS 16.0. Statistical significance for the ROI analyses was defined as $P < 0.05$. To correct for the likelihood of false positives, multiple comparisons were corrected using the FDR method of Benjamini and Hochberg to adjust the P values from the Pearson correlation analyses (Benjamini and Hochberg, 1995).

Authors’ contribution

Qiang Li undertook the MRI data analyses and wrote the manuscript; Yarong Wang wrote the protocol; Yi Zhang, Wei Li, Weichuan Yang, Jia Zhu, Ning Wu, Haifeng Chang Ying Zheng, Kai Yuan and Jixin Liu collected the clinical and MRI data and conducted the data analyses; and Jie Tian, Wei Wang, Wei Qin and Liyan Zhao contributed to the study design. All authors critically reviewed and have approved the final manuscript for publication.

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