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Predicting subsequent relapse by drug-related cue-induced brain activation in heroin addiction: an event-related functional magnetic resonance imaging study

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ABSTRACT

Abnormal salience attribution is implicated in heroin addiction. Previously, combining functional magnetic resonance imaging (fMRI) and a drug cue-reactivity task, we demonstrated abnormal patterns of subjective response and brain reactivity in heroin-dependent individuals. However, whether the changes in cue-induced brain response were related to relapse was unknown. In a prospective study, we recruited 49 heroin-dependent patients under methadone maintenance treatment, a gold standard treatment (average daily dose 41.8 ± 16.0 mg), and 20 healthy subjects to perform the heroin cue-reactivity task during fMRI. The patients' subjective craving was evaluated. They participated in a follow-up assessment for 3 months, during which heroin use was assessed and relapse was confirmed by self-reported relapse or urine toxicology. Differences between relapsers and non-relapsers were analyzed with respect to the results from heroin-cue responses. Compared with healthy subjects, relapsers and non-relapsers commonly demonstrated significantly increased brain responses during the processing of heroin cues in the mesolimbic system, prefrontal regions and visuospatial-attention regions. However, compared with non-relapsers, relapsers demonstrated significantly greater cue-induced craving and the brain response mainly in the bilateral nucleus accumbens/subcallosal cortex and cerebellum. Although the cue-induced heroin craving was low in absolute measures, the change in craving positively correlated with the activation of the nucleus accumbens/subcallosal cortex among the patients. These findings suggest that in treatment-seeking heroin-dependent individuals, greater cue-induced craving and greater specific regional activations might be related to reward/craving and memory retrieval processes. These responses may predict relapse and represent important targets for the development of new treatment for heroin addiction.

Keywords Craving, fMRI, heroin addiction, relapse.

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INTRODUCTION

Heroin addiction is a complex disorder of the brain, involving both affective and cognitive processes, characterized by a compulsive drive to take drugs regardless of serious negative consequences (Li & Sinha 2008). Despite the fact that most heroin-dependent individuals are willing to quit and that there are various heroin addiction treatments such as methadone maintenance treatment (MMT), which is deemed as an effective treatment for heroin addiction (Preston, Umbricht & Epstein 2000), relapse rates remain high. Studies showed that the

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relapse rate is 70 percent once patients left MMT (Farrell *et al*. 1994). One major situation often inducing relapse is the confrontation with heroin-related cues that have been regularly associated with heroin consumption. The conditioned cues can evoke heroin craving or relapse even without the presence of heroin itself. Note that craving for heroin is often denied by detoxified and treated heroin-dependent individuals, although they still show high relapse rates. There is also a study demonstrating that responses to drug-related cues that occur before craving rather than subjective craving itself may have better predictive value in terms of relapse (Tiffany & Carter 1998). To date, there are few neuro-imaging studies assessing cue-induced brain responses that predict relapse in heroin addiction. Neuro-imaging techniques hold the potential to examine whether any specific pattern of brain responses to drug-related cues can predict treatment outcome and, more specifically, relapse to drug use (Kosten *et al*. 2006). Developing a better understanding of neurobiological mechanisms underlying heroin cue-reactivity and developing the means to identify relapse-vulnerable individuals would possibly reduce relapse rates and relative morbidity and mortality.

Exposure to heroin-related versus neutral cues activates a wide range of brain regions, including mesolimbic system, prefrontal and visuospatial-attention regions such as nucleus accumbens (NAc), subcallosal cortex (SCC), amygdala, hippocampus, caudate, anterior cingulate cortex (ACC), medial prefrontal cortex, dorsolateral frontal cortex (DLPFC), orbitofrontal cortex (OFC), and temporal and parietal regions, as well as cerebellum (Daglish *et al*. 2001; Langleben *et al*. 2008; Zijlstra *et al*. 2009; Li *et al*. 2012, 2013). However, it is not clear whether these brain activations are closely related to subsequent relapse among treated heroin-dependent individuals. As drug addiction is a complex disorder of the brain, involving different networks such as the reward circuit (NAc, ventral tegmental area and ventral pallidum), conditioning/memory circuit (amygdala, medial OFC, hippocampus and dorsal striatum), executive control circuit (DLPFC, ACC and lateral OFC) and motivation/drive circuit (medial OFC, ventral ACC, ventral tegmental area, substantia nigra, dorsal striatum and motor cortex) (Volkow *et al*. 2011), it is unknown which circuit plays a more important role in relapse. The reward circuit (mainly including NAc) is viewed as an essential structure during the development of drug craving and likeliness to relapse (Filbey *et al*. 2009). Recently, there is a study reporting that heroin craving and relapse could be prevented with a memory retrieval-extinction procedure (Xue *et al*. 2012). Therefore, the importance of the memory circuit is also highlighted.

In the present study, we recruited 49 heroin-dependent patients and 20 healthy control subjects for functional magnetic resonance imaging (fMRI) of the event-related cue-reactivity task, a behavioral paradigm reliably used to examine cue-induced brain response (Wang *et al*. 2011; Li *et al*. 2012, 2013). The aim of our study was to assess the relationship between subjective heroin craving and brain response in heroin-dependent individuals when exposed to heroin-related cues and relapse during a 3-month follow-up period. We hypothesized that brain reactivity during a heroin-related cue-reactivity task can be used to predict relapse in heroin-dependent individuals. Specifically, we hypothesized that relapsing heroin-dependent patients relative to non-relapsing individuals show greater craving for heroin and more intense brain activation in reward-related and memory-related brain regions.

METHODS AND MATERIALS

Subjects

The present study was mainly among heroin-dependent patients under MMT in Baqiao MMT clinic, Xi'an, China, with a 3-month follow-up. Participants included 49 heroin-dependent male patients under MMT and 20 male healthy control individuals (Table 1). All of the subjects were smokers. Inclusion criteria for heroin-dependent patients were (1) DSM-IV criteria for heroin addiction for at least 1 year; (2) being under MMT for at least 6 months with a stable dose for at least 1 month; and (3) being right-handed. Exclusion criteria for all of the subjects were (1) use of cocaine or other illegal drug use except for heroin; (2) current or past psychiatric illness other than heroin and nicotine dependence; (3) neurological signs and/or history of neurological disease; (4) history of head trauma; (5) history of cardiovascular or endocrine disease; (6) current medical illness or recent medicine use; (7) presence of magnetically active objects in the body; and (8) claustrophobia or any other medical condition that would preclude the patient from lying in the MRI scanner for approximately 40 minutes. The Beck Depression Inventory II (BDI) (Beck *et al*. 1996) and Hamilton Anxiety Scale (HAMA) (Hamilton 1959) were used to evaluate the severity of depression and anxiety symptoms, respectively. All aspects of the research protocol were reviewed and approved by the ethics committee of Tangdu Hospital. All subjects provided written informed consent to participate in this study.

Design and procedure

We utilized a previously established event-related fMRI design in this study (Wang *et al*. 2011; Li *et al*. 2012, 2013). There were 48 trials in all, consisting of 24 heroin-related cues and 24 neutral cues. The heroin-related cues included pictures of heroin injection,

Table 1 Demographic and clinical characteristics of participants.

Characteristics	*Controls* (n = *20*)	*Relapsers* (n = *23*)	*Non-relapsers* (n = *21*)	*Group differences*	
Age	35.2 ± 7.0	31.3 ± 6.5	39.1 ± 7.6	$F = 6.75$	$P = 0.002$[a]
Years of education	10.0 ± 2.3	9.5 ± 2.3	9.2 ± 1.9	$F = 0.84$	$P = 0.44$
Cigarettes (per day)	13.7 ± 4.9	18.3 ± 7.2	21.8 ± 9.3	$F = 6.21$	$P = 0.004$[b]
BDI scores	3.1 ± 4.4	8.8 ± 9.3	10.0 ± 8.3	$F = 4.76$	$P = 0.02$[b]
HAMA scores	2.9 ± 3.9	7.4 ± 8.5	8.4 ± 8.7	$F = 3.17$	$P = 0.05$[b]
Duration of heroin use (months)	NA	69.2 ± 68.5	92.3 ± 70.5	$t = -1.19$	$P = 0.24$
Average heroin dose (g/day)	NA	0.5 ± 0.4	0.6 ± 0.6	$t = -0.91$	$P = 0.37$
Total heroin dose (g)	NA	1130.5.2 ± 1693.2	1151.8 ± 1229.6	$t = 0.05$	$P = 0.96$
Duration of MMT (months)	NA	18.3 ± 11.5	25.5 ± 17.3	$t = -1.63$	$P = 0.11$
Average methadone dose (mg/day)	NA	41.4 ± 14.0	41.0 ± 18.5	$t = -0.09$	$P = 0.93$
Total methadone dose (mg)	NA	23 269.5 ± 16 114.8	32 485.4 ± 32 130.3	$t = -1.22$	$P = 0.23$

[a]Relapsers < non-relapsers, $P < 0.05$; controls versus relapsers, no significant difference; controls versus non-relapsers, no significant difference. [b]Controls < relapsers, controls < non-relapsers, $P < 0.05$; relapsers versus non-relapsers, no significant difference. The total and average heroin dose was self-reported at baseline by heroin-dependent individuals.

preparation and paraphernalia, and the neutral cues included pictures of household objects or chores. All of the cues were projected onto a mirror fixed on the scanner head coil and were presented in a pseudo-randomized order with E-Prime 2.0 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Picture cues were presented for 2 seconds with a variable 4- to 12-second inter-stimulus interval (mean = 8 seconds), during which a white cross hair with black background was displayed. The task began with a 10-second dummy scan followed by the first cue (heroin-related or neutral cue) and experimental scanning. The total task lasted for 490 seconds. Participants were placed in the scanner in a supine position using a foam head holder to lessen motion. Earplugs were used to reduce scanner noise. No use of caffeine, tea, alcohol and any other drug or medicine was allowed 12 hours prior to the time of the MRI scan.

For heroin-dependent subjects, subjective heroin craving was evaluated by a 0–10 visual analog scale (Wang *et al.* 2011; Li *et al.* 2012, 2013) using the question, 'To what extent do you feel the urge to use heroin?' (0 for the *least craving* and 10 for the *strongest craving*). Craving ratings were acquired before and shortly after each fMRI scan. Heroin-dependent subjects were given a 'talkdown' to reduce heroin craving or subjective withdrawal symptoms after the fMRI scan, which may have been induced by heroin-related cues.

Longitudinal clinical follow-up

The procedures of longitudinal follow-up were similar to those described in Fatseas *et al.*'s (2011) study. All participants were given an appointment for a follow-up interview at 1, 2 and 3 months after the experimental session. Reminders were sent 3 days before each appointment. Heroin use was assessed at each follow-up appointment by the follow-up interview and urine screen. We used a sensitive method to capture any change in substance use patterns during the follow-up compared with the baseline evaluation. Substance use outcome was evaluated using both measures for heroin use and continuous measures for other substances (Fatseas *et al.* 2011). We thought that any heroin use and/or the increase of other substances (such as alcohol) used might be a symptom of relapse among MMT patients. Participants were considered relapsers if at any time during the 3-month follow-up period (1) they had used heroin defined by positive urine screen and/or self-reports of heroin use and (2) if they increased the number of days of self-reported use for other substances by at least 50 percent compared with the baseline evaluation (Fatseas *et al.* 2011). In addition, patients provided permission to contact people close to them who had knowledge of their heroin use, to get indirect information in the event that the patients were lost to follow-up. The assessors had no idea of cue-induced responses when the follow-up data were collected.

MRI data acquisition

All imaging data were acquired on a 3 T MRI scanner (GE Signa Excite HD, Milwaukee, WI, USA). The subjects underwent 'mock scans' for 1 minute prior to formal experimental scanning. This session served to familiarize subjects with the scanning environment. Following the mock scanning session, single-shot gradient-echo echo-planar imaging was used to acquire 240 T2*-weighted image volumes. For each volume, 32 axial slices covering the whole brain were acquired with the following parameters: repetition time = 2000 milliseconds, echo time = 30 milliseconds, flip angle = 90°, matrix = 64 × 64, field of view = 256 × 256 mm^2, slice thickness = 4 mm, gap = 0 mm, spatial resolution = 4 × 4 × 4 mm^3. To facilitate co-registration of the fMRI data

in standard space, a 166-slice high-resolution fast spoiled gradient-echo 3D T1-weighted image was also collected with the following parameters: repetition time = 7.8 milliseconds, echo time = 3.0 milliseconds, matrix = 256 × 256, field of view = 256 × 256 mm^2, slice thickness = 1 mm, spatial resolution = 1 × 1 × 1 mm^3. The structural data were carefully checked by an experienced radiologist to assure that there were no structural abnormalities.

Data analysis

The fMRI data analysis was conducted with SPM8 software (http://www.fil.ion.ucl.ac.uk/spm). Images were slice-time corrected, motion corrected, registered to the fast spoiled gradient-echo 3D T1-weighted images and then normalized to a standard SPM T1 template. The images were interpolated to 3-mm isotropic voxels and spatially smoothed (Gaussian kernel of 6-mm full width at half maximum). Subjects with excessive head motion (more than 1.5 mm in translation or 1.5° in rotation) were excluded from the analysis. The fMRI data were filtered using a high-pass filter and cut-off at 128 seconds. A statistical model for each subject was computed by applying a canonical response function. 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 versus neutral cues contrast which would reveal brain activities related to processing of heroin-related cues (Franklin *et al.* 2007). Because our main focus was on the difference between relapsers and non-relapsers, only using the healthy subjects as negative controls, we directly compared the different groups of subject (heroin-dependent individuals versus healthy controls, relapsers versus healthy controls, non-relapsers versus healthy controls and relapsers versus non-relapsers) using voxel-wise random effects two-sample *t*-tests to identify regions in which brain response to heroin-related > neutral cues differed between two groups. The age, index of smoking behavior, BDI scores and HAMA scores were taken as covariates into the test. In addition, the daily methadone dose and MMT duration were included as covariates in the analysis between the relapsers and non-relapsers. The significance threshold was set at $P < 0.05$, corrected for multiple comparison using AFNI Alphasim via Monte Carlo simulation correction program (Cox 1996).

For all of the heroin-dependent participants, the region of interest (ROI)-based correlation analyses were conducted to assess the relationship between craving change and brain activation intensity between viewing heroin-related and neutral cues. We chose the peak coordinate voxels of each differential cluster observed between the relapser and non-relapser groups as centers of the sphere-shaped ROIs (radius = 3 mm). The raw data within the ROIs of the heroin-dependent individuals were extracted and Pearson correlation analysis was conducted. To explore whether the intensity of heroin-related cue-induced brain response would be related to the period between cue exposure and relapse, we performed further Pearson correlation analysis to examine the predictors in relation to the time of relapse. The daily methadone dose, MMT duration, age, index of smoking behavior, BDI scores and HAMA scores were taken as covariates into the correlation analyses. The significance threshold was set at $P < 0.05$

RESULTS

Sample characteristics

Of the 69 participants who completed the MRI scan, four patients did not complete follow-up and were not included in the relapse analyses. Data from one patient were discarded due to a scanning artifact, leaving 44 patients and 20 usable healthy control subjects. According to our defined model of relapse, 23 (52.3 percent) patients were considered as relapsers. Nine patients reported using heroin and/or had a positive screening for opiates at 1-month follow-up. Eight patients at the 2-month follow-up and six patients at the 3-month follow-up reported using heroin and/or had a positive screening for opiates. During the 3-month follow-up, the relapsers reported times of relapse averaged 2.4 ± 1.9 and dose of heroin used averaged 0.6 ± 0.6 g. There were no patients who had significantly increased other substance (alcohol) use during the follow-up phase. There were no differences between relapsers and non-relapsers with respect to demographical data, drug use and psychiatric symptoms, except for age (Table 1).

Craving

For the subsequent relapser group, the subjective craving scores before and after cue exposure and change in craving were 1.6 ± 1.8, 1.8 ± 2.1 and 0.2 ± 1.8, respectively. For the non-relapser group, the subjective craving scores before and after cue exposure and change in craving were 1.0 ± 1.2, 0.5 ± 0.9 and –0.5 ± 1.3, respectively. Meanwhile, the subsequent relapser group demonstrated significantly higher craving scores after cue exposure relative to the non-relapser group ($t = 2.78$, $P = 0.01$). No significant difference in the craving score before cue exposure ($t = 1.22$, $P = 0.23$) and craving change ($t = 1.52$, $P = 0.14$) was found between the two groups. No significant change in the craving score before and after cue exposure was found for the relapse and non-relapser groups, respectively ($t = –0.47$, $P = 0.64$; $t = –1.86$, $P = 0.08$) (Fig. 1).

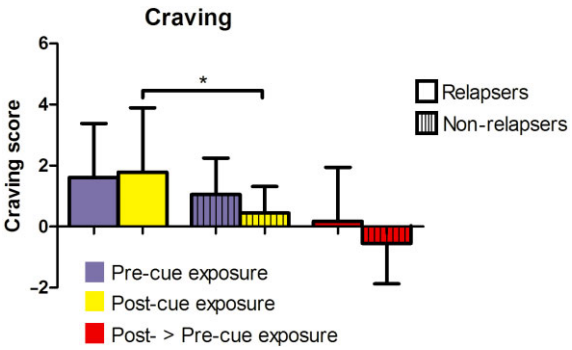

Figure 1 Changes in subjective craving according to heroin-related cue exposure in heroin-dependent groups. *Significant difference ($P < 0.05$). Relapsers showed higher post-cue exposure craving for heroin than non-relapsers ($P = 0.01$)

fMRI results

Heroin-dependent individuals versus healthy controls: heroin-related > neutral cues

Compared with the healthy control group, the heroin-dependent group demonstrated significantly increased brain responses during the processing of heroin-related cues in the bilateral NAc/SCC, cerebellum, caudate, putamen, pallidum, DLPFC, OFC, parahippocampal gyrus, inferior parietal lobule, precuneus, inferior occipital gyrus, and inferior temporal gyrus, pons, and left ACC, MPFC, midbrain, superior parietal lobule, superior temporal gyrus, and right middle cingulate gyrus and fusiform (Table 2, Fig. 2 and Supporting Information Fig. S1). No significantly greater brain response for the healthy control group relative to the heroin group was found.

Table 2 Activated brain regions for the heroin-dependent group compared with control group in response to heroin-related > neutral cues.

Brain regions		*Brodmann's area*	*Peak location*			*Peak t-score*	*Voxel number*
			x	*y*	*z*		
NAC/SCC	R/L	25	−3	15	−12	4.33	93
Cerebellum	R/L	–	0	−60	−36	4.92	41
	R		12	−87	−27	4.23	19
Caudate	L	–	−12	9	5	4.21	42
	R	–	18	19	9	4.95	76
Putamen	L	–	−18	10	−2	3.87	37
	R	–	30	−6	6	4.17	29
Midbrain	L	–	−9	−27	−12	4.95	10
Pallidum	L	–	−6	0	−12	4.68	29
	R	–	12	−3	−12	5.30	15
ACC	L	24,32	−9	45	0	4.93	57
Middle cingulate gyrus	R	23	6	−9	33	3.86	10
DLPFC	L	48	−42	9	24	4.36	29
	R	44,48	45	9	21	5.42	75
MPFC	L	32	−9	30	39	4.37	44
OFC	L	11	−15	15	−21	5.12	39
	R	45	51	24	0	3.99	33
Parahippocampal gyrus	L	28	−11	−1	−20	3.95	20
	R	28	14	−1	−18	4.56	19
Superior parietal lobule	L	7	−24	−72	48	4.42	13
Inferior parietal lobule	L	40	−42	−51	54	4.75	39
	R	40	42	−48	48	4.52	47
Precuneus	L	30	−6	−54	12	4.40	35
	R		9	−51	12	3.79	10
Fusiform	R	37	33	−33	−24	4.95	27
Pons	R/L	–	0	−33	−27	4.37	18
Inferior occipital gyrus	L	18	−18	−93	−12	4.23	13
	R	19	33	−81	−15	4.12	15
Superior occipital gyrus	L	18	−15	−96	21	5.50	16
Inferior temporal gyrus	L	37	−57	−63	−9	4.32	45
	R	37	60	−63	−9	4.59	31
Superior temporal gyrus	L	38	−34	6	−19	4.18	10

ACC = anterior cingulate cortex; DLPFC = dorsolateral prefrontal cortex; L = left; MPFC = medial prefrontal cortex; NAc/SCC = nucleus accumbens/subcallosal cortex; OFC = orbitofrontal cortex; R = right.

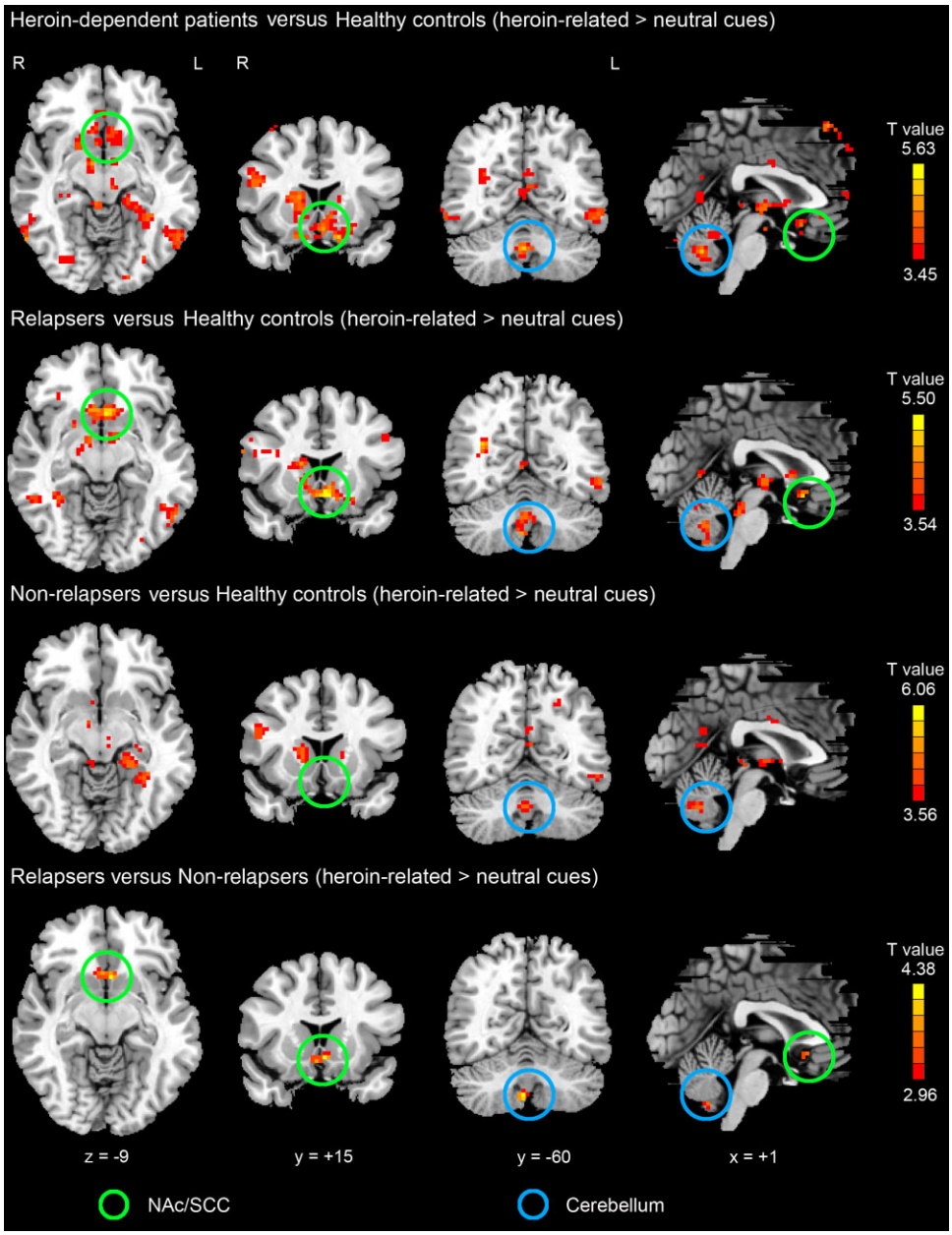

Figure 2 The differences relating to the 'heroin-related > neutral cues' contrast between heroin-dependent patients and healthy controls, between relapsers and healthy controls, between non-relapsers and healthy controls, and between relapsers and non-relapsers ($P < 0.05$, corrected for Monte Carlo simulations correction). L = left; NAc/SCC = nucleus accumbens/subcallosal cortex; R = right

Relapsers versus healthy controls: heroin-related > neutral cues

Compared with the healthy control group, the subsequent relapser group demonstrated significantly increased brain responses during the processing of heroin-related cues in the bilateral NAc/SCC, caudate, DLPFC, cerebellum, left ACC, MPFC, midbrain, superior parietal lobule, inferior parietal lobule, precuneus, inferior and superior temporal gyrus, and right pallidum and pons (Fig. 2 and Supporting Information Table S1 and Fig. S2). No significantly greater brain response for the healthy control group relative to the subsequent relapser group was found.

Non-relapsers versus healthy controls: heroin-related > neutral cues

Compared with the healthy control group, the non-relapser group demonstrated significantly increased brain responses during the processing of heroin-related cues in the bilateral caudate, putamen, pallidum, DLPFC,

parahippocampal gyrus, cerebellum, inferior parietal lobule, and left ACC, hippocampus, midbrain, precuneus, superior parietal lobule and right middle cingulate gyrus, precentral gyrus (Fig. 2 and Supporting Information Table S2 and Fig. S3). No significantly greater brain response for the healthy control group relative to the non-relapser group was found.

Relapsers versus non-relapsers: heroin-related > neutral cues

Compared with the non-relapser group, the subsequent relapser group demonstrated significantly increased brain responses during the processing of heroin-related cues in the bilateral NAc/SCC and cerebellum. No significantly greater brain response for the non-relapser group relative to the subsequent relapser group was found (Table 3 and Fig. 2).

Correlation results

For heroin-dependent patients, a significant positive correlation between changes in craving and brain activity to heroin-related cues was found for the NAC/SCC ($r = 0.30$, $P = 0.04$) (Fig. 3). No significant correlations were found between the drug cue-induced brain activity and the time period between drug cue exposure and relapse.

Table 3 Activated brain regions for the relapser compared with non-relapser group in response to heroin-related > neutral cues.

Brain regions		*Brodmann's area*	*Peak location*			*Peak* t-*score*	*Voxel number*
			x	*y*	*z*		
NAc/SCC	R/L	25	−3	15	−8	4.38	14
Cerebellum	R/L	–	3	−60	−48	4.29	14

L = left; NAc/SCC = nucleus accumbens/subcallosal cortex; R = right.

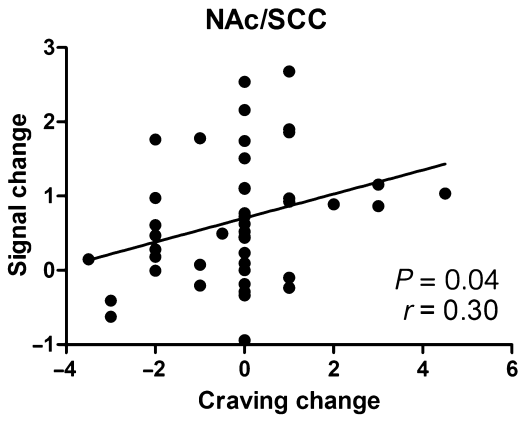

Figure 3 The correlation map between craving change and signal amplitude of nucleus accumbens/subcallosal cortex (NAc/SCC) relating to the 'heroin-related > neutral cues' contrast among the heroin-dependent patients (r = correlation coefficient; P = P-value)

DISCUSSION

To the best of our knowledge, this is the first neuroimaging study to assess brain responses that may predict relapse in heroin addiction. The present findings demonstrated that increased brain response in the NAc/SCC and cerebellum during processing of heroin-related cues is associated with the prospective relapse in treated heroin-dependent individuals. Our findings confirmed our hypothesis and suggested that greater heroin craving and brain activation in reward/craving-related and memory-related brain regions is associated with relapse. More generally, it also contributed to a growing body of literature in which drug cue-based baseline neuro-imaging evaluations are employed to predict future relapse (Janes *et al.* 2010; Beck *et al.* 2012; Moeller *et al.* 2012; Seo *et al.* 2013).

Compared with the healthy control group, the subsequent relapser and non-relapser groups commonly demonstrated significantly increased brain responses during the processing of heroin-related cues in the mesolimbic system (caudate, pallidum), prefrontal regions (ACC and DLPFC), visuospatial-attention regions (precuneus, inferior parietal lobule and superior parietal lobule), midbrain and cerebellum. These results were in line with our previous research (Wang *et al.* 2011; Li *et al.* 2012, 2013) and others' studies (Sell *et al.* 1999; Daglish *et al.* 2001; Langleben *et al.* 2008, 2014; Yang *et al.* 2009; Zijlstra *et al.* 2009; Walter *et al.* 2014) showing an enhanced cue-induced brain response in these areas. The ACC and DLPFC have been demonstrated to be involved in reward prediction, decision making, inhibitory control and salience attribution (Garavan *et al.* 2000; Miller 2000; Watanabe *et al.* 2002; Kalivas & Volkow 2005). The midbrain and pallidum have been demonstrated to play a role in reward (Wang *et al.* 2007). The precuneus and inferior and superior parietal lobules have been demonstrated to be involved in visuospatial attention (Due *et al.* 2002; Spanagel 2003). All of the results indicated that heroin-related cues can induce enhanced salience attribution among the heroin-dependent patients under MMT.

However, more importantly, compared with the group of non-relapsers, the group of relapsers demonstrated significantly increased brain responses to heroin-related > neutral cues in the bilateral NAc/SCC and cerebellum. The NAc/SCC is a highly dopamine-innervated brain region and plays an important role in the function of reward, subjective euphoria and craving (Breiter *et al.* 1997; Kilts *et al.* 2001). Pre-clinical studies in animals indicate that this region plays a key role in Pavlovian

conditioning (Parkinson *et al.* 1999), control of instrumental behavior by Pavlovian cues (Corbit, Muir & Balleine 2001) and behavior of drug seeking by drug-paired cues (Ito, Robbins & Everitt 2004). Drugs of abuse lead to excessive dopamine neurotransmission in the ventral striatum where the NAc/SCC is located (Kalivas & Stewart 1991). The increase of dopamine neurotransmission in the NAc/SCC could even be induced by cues related to drugs such as amphetamine (Boileau *et al.* 2007). On the contrary, animal studies have demonstrated that the procedure of deep brain stimulation (DBS) of NAc is effective to modulate the behavior of alcoholism (Knapp *et al.* 2009), cocaine seeking (Vassoler *et al.* 2008) and opiate addiction (Liu *et al.* 2008). Further, clinical case reports have also shown that craving and risk of relapse in smoking (Kuhn *et al.* 2009), alcoholism (Heinze *et al.* 2009) and heroin addiction (Gao *et al.* 2003; Wu *et al.* 2010) can be promisingly decreased by means of DBS or ablation of the NAc. Our fMRI finding, showing a positive correlation between heroin-related cue-induced craving change and activation in the NAc/SCC among heroin-dependent patients, further supports the NAc/SCC in the human, with a potential role in incentive as well as expectation of reward. The significantly increased NAc/SCC activity and craving to heroin-related cues among the relapsers relative to non-relapsers suggested that, although under stable MMT, the more NAc/SCC activity and craving to the heroin-related cues heroin-dependent patients showed, the more relapse vulnerability they had. Therefore, our findings further highlighted the key role of NAc/SCC in heroin relapse.

The cerebellum had once been viewed only as a mediator of motor functions (Stein & Glickstein 1992). However, there is growing evidence demonstrating that the cerebellum also plays a role in memory retrieval and learning during performance of higher order cognitive tasks such as drug cue-induced craving (Buckner *et al.* 1996; Yacubian *et al.* 2007). The cocaine craving studies demonstrated that the learned memory associations of drug use are mediated by the cerebellum (Hariri *et al.* 2005; Zubieta *et al.* 2005). Recently, an animal study (Carbo-Gas *et al.* 2014) demonstrated that olfactory stimulus preference was directly associated with cFos expression in cells at the apical region of the granule cell layer of the cerebellar vermis in cocaine-addicted mice. The results also suggested that the cerebellum might be an important part of the neural circuits involved in generating, maintaining and/or retrieving drug memories. Our findings, showing greater heroin-related cues induced cerebellar activation in relapsers relative to non-relapsers, suggested that the abnormal memory retrieval function of cerebellum may play an important role in relapse among treated heroin-dependent patients. As is known, drug use and relapse involve learned associations between drug-associated environmental cues and drug effects (Xue *et al.* 2012). The significantly increased cerebellar activation to heroin-related cues among the relapsers relative to non-relapsers suggested that the cue-induced cerebellar activity might also be a potential biomarker of relapse. It further suggested that the learned memory of drug use experience plays an important role in subsequent relapse even when heroin-dependent patients are under stable MMT. However, more fMRI studies are needed to understand the memory retrieval role of the cerebellum in heroin addiction.

The current findings have some clinical implications. Our findings suggest that future therapies for heroin addiction should assess cue-induced brain responses prior to treatment as an indicator of relapse potential. Moreover, changes in drug cue-induced brain responses after a certain therapy may be a potentially reliable marker of treatment efficacy. Therapies for heroin addiction that would block such responses to heroin-related cues would presumably reduce vulnerability of relapse. Recently, Langleben *et al.* (2014) and Walter *et al.* (2014) demonstrated significantly changes in the patterns of brain response to drug-related cues after administration of naltrexone and pharmaceutical heroin (diacetylmorphine). These two studies further confirmed the potential value of drug cue-response measures in the evaluation of the efficacy of therapies. In addition, our findings support the notion that NAc/SCC and cerebellum may be targets for the development of addiction treatment.

Some caveats apply to this study. First, the heroin-dependent individuals averaged more than 5 years of heroin use and were all relapsers in the past. Therefore, patients who did not relapse at 3 months would most likely relapse to heroin use with a longer follow-up period. These considerations suggest that the present cohort may not be ideal to examine neural predictors of relapse given neural-plastic adaptations as a result of long-term heroin exposure. Second, these heroin-dependent patients under long-term MMT focused on relapse prevention and had a stable dose of methadone treatment. The effect of methadone may influence our results. Various laboratory- and clinical-based studies have demonstrated that methadone plays a role in suppressing heroin craving, alleviating withdrawal symptoms and, in turn, reducing the relapse rates (Kreek 2000). However, the phenomena of relapse still exist (Fatseas *et al.* 2011).

In summary, we found that increased cue-induced activation in the NAc/SCC and cerebellum can predict subsequent relapse among heroin-dependent individuals. Our findings shed light on the development of treatment targeting the NAc/SCC and cerebellum for preventing relapse in heroin addiction.

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Disclosure/Conflict of Interest

All of the authors state that they have no conflicts of interest and have nothing to declare.

Authors Contribution

WW was responsible for the study design. JZ, YZ, DZ, LW, YL, XY, HC, MF and ZL contributed to the acquisition of fMRI and demographical data. HW and QL performed the data analysis. WL and YZ assisted with data analysis and interpretation of findings. QL drafted the manuscript. YL, JT, YW and MG provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 The differences relating to the 'heroin-related > neutral cues' contrast between heroin-dependent patients and healthy controls ($P < 0.05$, corrected for Monte Carlo simulations correction). R, right, L, left

Figure S2 The differences relating to the 'heroin-related > neutral cues' contrast between relapsers and healthy controls ($P < 0.05$, corrected for Monte Carlo simulations correction). R, right, L, left

Figure S3 The differences relating to the 'heroin-related > neutral cues' contrast between nonrelapsers and healthy controls ($P < 0.05$, corrected for Monte Carlo simulations correction). R, right, L, left

Table S1 Activated brain regions for the relapser compared with control group in response to heroin-related > neutral cues

Table S2 Activated brain regions for the nonrelapsers compared with control group in response to heroin-related > neutral cues