Brain opioid receptor binding in early abstinence from opioid dependence

Positron emission tomography study

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Background Although opioid receptor function in humans is clearly reduced during opioid dependence, what happens to the receptor in early abstinence is not understood.

Aims This study sought to examine changes in opioid receptor availability in early abstinence from opioid dependence.

Method Ten people with opioid dependence who had completed in-patient detoxification and 20 healthy controls underwent [11C]-diprenorphine positron emission tomography. Clinical variables were assessed with structured questionnaires. Opioid receptor binding was characterised as the volume of distribution of [11C]-diprenorphine using a template of predefined brain volumes and an exploratory voxel-by-voxel analysis.

Results Compared with controls, participants with opioid dependence had increased [11C]-diprenorphine binding in the whole brain and in 15 of the 21 a priori regions studied.

Conclusions This study suggests that opioid receptor binding is increased throughout the brain in early abstinence from dependent opioid use. These data complement the findings in cocaine and alcohol dependence.

Declaration of interest None. Funding detailed in Acknowledgements.

METHOD

Participants We recruited ten people with opioid dependence (8 male, 2 female; mean age 31.7 years, s.d. = 6.3, range 25–45) undergoing in-patient detoxification at Bristol Specialist Drug Service (Bristol, UK). All fulfilled DSM-IV criteria for opioid dependence (American Psychiatric Association, 1994) prior to detoxification but were excluded if they fulfilled diagnostic criteria for another current Axis I disorder (excluding nicotine dependence). The participants were in their 10th day of a lofexidine-assisted in-patient detoxification regime from methadone and were free of opioids or any other drug of misuse as confirmed by urinalysis at the time of scanning. They were typically long-standing opioid users and had been on methadone for a mean of 6.9 years (s.d. = 5.2, range 2–17 years); the mean dose at the start of detoxification was 31 mg/day (s.d. = 12, range 15–50). The patients had a history of using a variety of other drugs prior to admission (Table 1); all but one participant continued to use heroin in addition to their prescribed methadone in the month prior to detoxification, half were using ‘crack’ cocaine prior to detoxification and all were tobacco smokers. All participants underwent the standard detoxification regimen under the care of the clinical team, independent of the investigators.

Twenty healthy people (18 male, 2 female; mean age 34.8 years, s.d. = 8.3, range 25–48) with no history of dependence on any drug except nicotine (all current non-smokers) were recruited as controls. Controls had no history of serious psychiatric or medical disorder as determined by clinical interview. They were recruited for this and other studies to form a common pool, to avoid unnecessary duplication and radiation exposure. Therefore, only 8 of the 20 controls completed all the same questionnaires as participants with opioid dependence. The control group for this study was selected to match the age range of participants with opioid dependence.

Local research ethics committees and the UK Administration of Radioactive Substances Advisory Committee approved all procedures and experimental protocols. After full explanation of the study procedures, volunteers gave written informed consent.

Clinical measures Subjective measures included the Adjective Checklist (Jasinski, 1997), which measures effects of opioid agonists (16 items) and opioid withdrawal symptoms (21 items), and the 49-item short form of the Addiction Research Centre Inventory (ARCI), which is scored for euphoria, dysphoria and sedation scales (Haertzen, 1970).

Objective evidence of withdrawal (Opiate Withdrawal Scale; OWS) was measured using an adaptation of the Kolb and Himmelsbach point system as previously described (Law et al, 1997). Drug craving was assessed using two tools; the 45-item...
Radioactivity in arterial blood was assayed in horizontal image planes (Jones, 1994) and reconstructed into 31 contiguous horizontal emission data were acquired over 90 min, with 10% uptake of the injected dose after 15 min. 

Heroin was given intravenously over 30 s. Dynamic PET scans were performed with in-house receptor parametric mapping software implemented in Matlab (Mathworks Inc., Natick, Massachusetts) (Gunn et al., 2002). Spectral analysis with individual metabolite corrected plasma input function takes account of any differences in tracer delivery between individuals or groups. The volume of distribution is the ratio of total free and bound tissue to free plasma ligand concentration at equilibrium and provides an index of receptor binding.

Image processing and statistical analysis

The dynamic PET scans were analysed to produce parametric images of ligand volume of distribution using spectral analysis with in-house receptor parametric mapping software implemented in Matlab (Mathworks Inc., Natick, Massachusetts) (Gunn et al., 2002). Spectral analysis with individual metabolite corrected plasma input function takes account of any differences in tracer delivery between individuals or groups. The volume of distribution is the ratio of total free and bound tissue to free plasma ligand concentration at equilibrium and provides an index of receptor binding.

We used Statistical Parametric Mapping (SPM2, Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, UK) running in Matlab to transform the volume of distribution images into a standard space as defined by the Montreal Neurological Institute (Evans et al., 1993) using a weighted-mean $^{11}$C-diprenorphine template created inhouse from the PET scans of seven healthy volunteers.

We performed two types of analysis, first using predefined volumes of interest and then using SPM2 for an exploratory analysis. Twenty-one regions for which we had an a priori hypothesis for increased opioid receptor availability based on previous studies were selected for comparison. These areas were the orbito-frontal cortex, anterior cingulate, ventral striatum (including the nucleus accumbens), dorsal striatum (including the caudate nucleus and putamen), thalamus, amygdala and periaqueductal grey matter. All these regions have been shown to play a role in the addiction process. The anterior cingulate cortex, orbitofrontal cortex, nucleus accumbens and amygdala are key in reward and motivation during drug-use from the evaluation of stimuli to reward-based decision-making and learning. The periaqueductal grey matter is an important element of the endogenous opioid system and is involved in conditioned processes in dependent drug use. Similarly the thalamus, caudate and putamen form part of the emotional reward neurocircuitry which has an important role in motivational factors and links to motor pathways, possibly being a route for the development of locomotor sensitisation with continued drug use (Kalivas & Volkow, 2005; Nutt et al., 2006).

We used statistical parametric mapping to transfer 73 standardised volumes of interest derived from a probabilistic atlas of brain images (Hamners et al., 2003) onto individual scans by inverting the deformations used to spatially normalise the images. The volume of distribution maps were sampled using the individualised atlas for every participant to generate mean volume of distribution values for each volume of interest. These values were then compared between groups using a two-tailed non-paired t-test – unequal variances were assumed. Pearson’s correlation statistics were used to assess the association of clinical variables with opioid receptor binding.

In addition, $^{11}$C-diprenorphine volume of distribution images were analysed on a voxel-by-voxel basis using SPM2. Spatially normalised parametric images were smoothed with a 12 mm kernel at full width half maximum. Mean differences between groups were interrogated using non-paired t-tests, and correlations between clinical variables and $^{11}$C-diprenorphine binding were explored using linear regression within the general linear model in SPM2. Proportional scaling was used to normalise global differences throughout. For regions where there existed an a priori hypothesis, results are reported as significant at a threshold of $P<0.05$ uncorrected. For all other areas familywise error correction was used.

### Table 1 Drug and alcohol use in the 30 days prior to scanning

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ever used (n=10)</th>
<th>Any use in past 30 days (n=10)</th>
<th>Days used in past 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heroin</td>
<td>10</td>
<td>9</td>
<td>14.7 (4.7)</td>
</tr>
<tr>
<td>Intravenous</td>
<td>10</td>
<td>7</td>
<td>12.7 (6.4)</td>
</tr>
<tr>
<td>Smoked</td>
<td>10</td>
<td>4</td>
<td>17.5 (3.9)</td>
</tr>
<tr>
<td>Cannabis</td>
<td>10</td>
<td>8</td>
<td>10.3 (7.7)</td>
</tr>
<tr>
<td>Crack</td>
<td>9</td>
<td>5</td>
<td>8.2 (5.4)</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>10</td>
<td>4</td>
<td>15.0 (6.0)</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>9</td>
<td>0</td>
<td>15.0 (6.0)</td>
</tr>
<tr>
<td>MDMA</td>
<td>8</td>
<td>0</td>
<td>15.0 (6.0)</td>
</tr>
<tr>
<td>LSD</td>
<td>10</td>
<td>0</td>
<td>15.0 (6.0)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>10</td>
<td>5</td>
<td>8.2 (6.3)</td>
</tr>
<tr>
<td>Nicotine</td>
<td>10</td>
<td>10</td>
<td>30 (s.d.)</td>
</tr>
</tbody>
</table>

MDMA, methylendioxymethamphetamine; LSD, lysergic acid diethylamide.
RESULTS

Clinical measures
At the time of scanning, there was no clinically significant opioid withdrawal as measured by the OWS (mean score 1.2, s.d. = 1.2, range 0–3). However the subjective ARCI and Adjective Checklist showed higher measures of withdrawal in participants with opioid dependence than in controls. They showed significantly higher state, but not trait, anxiety than controls on the day of their scan. Participants with opioid dependence scored significantly lower than controls on some measures of health (Table 2). On the personality questionnaires participants with opioid dependence scored significantly higher for psychoticism, extraversion, impulsiveness and venturesomeness than controls, but not for neuroticism or empathy.

All participants with opioid dependence reported craving on the HCQ (mean score 15.7, s.d. = 6.0) and modified OCDS (mean score 21.67, s.d. = 10.6), and the scores were highly correlated (r = 0.76, P < 0.018). Craving scores were comparable with our previous study of the same stage of detoxification where craving was elicited using an imagery-based procedure (Weinstein et al., 1997).

Image analysis
Participants with opioid dependence showed a significantly higher level of opioid receptor availability, as measured by global [11C]-diprenorphine volume binding for people with opioid dependence (Table 2). On the personality questionnaires participants with opioid dependence scored significantly higher for psychoticism, extraversion, impulsiveness and venturesomeness than controls, but not for neuroticism or empathy.

Clinical measures

<table>
<thead>
<tr>
<th>Test and measure</th>
<th>Opioid dependent (n=10)</th>
<th>Controls (n=8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Withdrawal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observer Withdrawal Scale</td>
<td>1.2 (1.2)</td>
<td>0.1 (0.4)</td>
<td>0.024</td>
</tr>
<tr>
<td>Adjective Checklist—withdrawal</td>
<td>20.8 (9.9)</td>
<td>5.0 (5.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjective Checklist—agonist effects</td>
<td>22.5 (9.4)</td>
<td>20.5 (5.1)</td>
<td>0.595</td>
</tr>
<tr>
<td>ARCI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Withdrawal effects</td>
<td>23.0 (9.9)</td>
<td>5.0 (5.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Euphoric effects</td>
<td>7.8 (3.4)</td>
<td>4.3 (2.8)</td>
<td>0.030</td>
</tr>
<tr>
<td>Agonist effects</td>
<td>22.5 (9.3)</td>
<td>20.5 (5.1)</td>
<td>0.595</td>
</tr>
<tr>
<td>Spielberger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSAI – state</td>
<td>43.7 (8.6)</td>
<td>32.9 (9.1)</td>
<td>0.014</td>
</tr>
<tr>
<td>STAI – trait</td>
<td>44.5 (11.5)</td>
<td>37.5 (10.6)</td>
<td>0.172</td>
</tr>
<tr>
<td>SF–36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General health</td>
<td>43.6 (19.5)</td>
<td>82.5 (14.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical function</td>
<td>80.2 (10.6)</td>
<td>96.9 (5.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Physical role limitation</td>
<td>45.0 (36.9)</td>
<td>96.9 (8.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Bodily pain</td>
<td>39.7 (17.9)</td>
<td>83.4 (19.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Emotional role limitation</td>
<td>43.3 (41.7)</td>
<td>87.5 (35.4)</td>
<td>0.030</td>
</tr>
<tr>
<td>Social functioning</td>
<td>52.5 (23.4)</td>
<td>87.6 (17.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Vitality</td>
<td>39.0 (16.5)</td>
<td>49.6 (16.5)</td>
<td>0.193</td>
</tr>
<tr>
<td>Mental health</td>
<td>43.8 (11.8)</td>
<td>45.8 (9.9)</td>
<td>0.699</td>
</tr>
<tr>
<td>EPQ–R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychoticism</td>
<td>11.0 (2.3)</td>
<td>5.2 (2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Extraversion/intraversion</td>
<td>18.9 (4.4)</td>
<td>13.7 (3.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>15.3 (5.1)</td>
<td>11.9 (8.2)</td>
<td>0.285</td>
</tr>
<tr>
<td>IVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impulsiveness</td>
<td>13.5 (4.5)</td>
<td>4.9 (2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Venturesomeness</td>
<td>12.3 (2.8)</td>
<td>8.0 (3.5)</td>
<td>0.010</td>
</tr>
<tr>
<td>Empathy</td>
<td>13.0 (4.0)</td>
<td>14.3 (3.1)</td>
<td>0.477</td>
</tr>
</tbody>
</table>

1. n = 10.
2. n = 9.

ARCI, Addiction Research Centre Inventory; SF–36, 36-item short form of the General Health Survey; EPQ–R, Eysenck Personality Questionnaire – Revised; IVE, Eysenck Impulsiveness Questionnaire.

Fig. 1 Global and a priori regional [11C]-diprenorphine binding for people with opioid dependence (■) and controls (□). *P < 0.05 unpaired t-test.
The re-detoxification daily methadone total dose (mg/kg) and duration of methadone use showed no association with either global or regional \[^{11}C\]-diprenorphine binding. Furthermore, no relationship with \[^{11}C\]-diprenorphine binding was found when the participants with opioid dependence were divided into two groups: short-term users (use for 10 years) and long-term users (use for more than 10 years). We found no significant effect for either alcohol or ‘crack’ cocaine on \[^{11}C\]-diprenorphine binding by comparing those that had used in the previous 30 days with those that had not. Of interest, although it did not achieve significance, was a trend towards a negative correlation between level of opioid use in the previous month, year or lifetime and global \[^{11}C\]-diprenorphine binding. There was no correlation between \[^{11}C\]-diprenorphine volume of distribution and craving, subjective opioid effects or withdrawal, or any of the personality variables.

Regional densities of \(\mu\) opioid receptors, as reported in post-mortem tissue (Pfeiffer et al., 1982), correlated with \[^{11}C\]-diprenorphine binding in each region for the whole group \((r=0.54, P=0.006)\), and when control and patient groups were analysed separately \((r=0.51, P=0.010\) and \(r=0.53, P=0.007\) respectively). There was no correlation between \[^{11}C\]-diprenorphine volume of distribution and \(\kappa\) opioid receptor \((\text{combined group } r=0.05, P=0.803)\) or \(\delta\) opioid receptor regional densities \((\text{combined group } r=0.31, P=0.141)\). No differences were apparent between the groups in these correlations.

An exploratory comparison of people with opioid dependence and controls using statistical parametric mapping showed a significant increase in \[^{11}C\]-diprenorphine binding only in the right fusiform and parahippocampal gyri \((\text{MNI coordinates } x=38\ mm, y=-26\ mm, z=-32\ mm, \text{cluster size} 594\ \text{voxels, peak } T=5.15, P=0.016\) familywise error corrected). No significant correlations were found using statistical parametric mapping between \[^{11}C\]-diprenorphine volume of distribution and any clinical variables. Using a statistical threshold of \(P=0.05\) uncorrected to investigate the \textit{a priori} areas confirmed the findings of the region of interest analysis.

**Opioid receptor availability in dependence**

There is limited preclinical research that helps to interpret the results of this study. Although it is clear that chronic opioid exposure leads to reduced opioid receptor function \((\text{tolerance}),\) the mechanisms through which this is achieved are not certain and may include receptor internalisation or reduced receptor–effector coupling (Williams et al., 2001). \textit{In vitro} studies have shown that chronic exposure to an opioid agonist can lead to a downregulation in opioid receptors (Goodman et al., 1996). However, this is not a consistent pattern in \textit{in vivo} studies, which have reported increases, decreases and no change in opioid receptors, depending on the paradigm used (Zadina et al., 1995). In humans, tolerance to opioid agonists is well characterised but there are virtually no data on brain opioid receptor imaging. We have previously demonstrated a dose-related reduction in opioid receptor function in people with opioid dependence who are on methadone maintenance by showing that they are less sensitive to the effects of an opioid agonist, hydromorphone (Melchar et al., 2003). However, in a parallel \[^{11}C\]-diprenorphine PET study, we found no difference in binding compared with a healthy control group, suggesting limited occupancy and no significant changes in receptor number (Melchar et al., 2005). This complements a study using \[^{18}F\]-cyclofoxy PET that also suggested that methadone requires only very low levels of opioid receptor occupancy for efficacy (Kling et al., 2000). Lastly, post-mortem studies of people with heroin dependence have shown inconsistent changes \((\text{reduction or no difference})\) in \(\mu\) opioid receptor density compared with healthy controls (Gabilondo et al., 1994; Ferrer-Alcon et al., 2004). These studies suggest that chronic opioid exposure might not alter opioid receptor availability and importantly not increase receptor availability.

**Increased \[^{11}C\]-diprenorphine binding**

The increase in \[^{11}C\]-diprenorphine binding reflects an increase in availability of opioid receptors to this PET tracer. Increased receptor affinity for the tracer could account for this increased availability, but there is no preclinical evidence that chronic opioid administration alters affinity. Therefore, the increase in \[^{11}C\]-diprenorphine binding might be due to: (a) an increase in opioid receptors during early abstinence from opioid drugs; (b) an increase in opioid receptor number that develops with the chronic use of an opioid agonist; \((c)\) a reduction in competition from endogenous opioids. We believe that it is most likely that our findings reflect a significant increase in opioid receptor number immediately following detoxification from opioids. We know that withdrawal and early abstinence is a time when the brain is under stress, and that such an increase might represent a neuroadaptive response. This would explain the similar findings after cocaine and alcohol dependence (Zubieta et al., 1996; Gorelick et al., 2005; Heinz et al., 2005).

Increased \[^{11}C\]-diprenorphine binding could also reflect increased opioid receptor availability as a result of suppression of endogenous opioid release. Preclinical evidence shows that chronic treatment with methadone does not alter the concentration or function of endogenous opioids, although later studies with other opioids and other drugs of misuse suggest that endogenous opioids play a role in craving or drug-seeking behaviour \((\text{for a review see Gerrits et al., 2003})\). Activation of the endogenous opioid system is associated with the regulation of emotions, physical and emotional pain (Ribeiro et al., 2005). A possibility is that the exogenous opioids used to alter emotions by people with opioid dependence might lead to suppression of the endogenous opioid system and consequently a compensatory upregulation of receptors. This would leave more receptors available for occupancy by \[^{11}C\]-diprenorphine in early abstinence.

We are not aware of any human studies describing the impact of chronic opioid

**DISCUSSION**

This is the first study, to our knowledge, that has studied opioid receptor binding using \[^{11}C\]-diprenorphine PET in people with opioid dependence undergoing recent detoxification. We found a significant increase in \[^{11}C\]-diprenorphine volume of distribution in the majority of \textit{a priori} regions of interest, although notably not in the nucleus accumbens or caudate nucleus. Exploratory statistical parametric mapping at a threshold for significance applying a full correction for multiple comparisons identified the right fusiform and right parahippocampal gyri as areas of significantly increased binding. At a lower threshold, the mapping confirmed the findings of the volume of interest analysis when applied to regions specified \textit{a priori}. 
agonist use on levels of endogenous opioids.

**Opioid system after abstinence from substances**

In addition to being the primary target for opioid drugs, the opioid neurotransmitter system is important in initiating and maintaining dependence on a variety of misused substances (Herz, 1997; Gerrits et al, 2003; Kreek et al, 2004). A number of recent neuroimaging studies in humans using the μ-selective agonist [11C]-carfentanil have reported elevations of tracer binding in early abstinence from cocaine and alcohol, which are associated with craving (Zubieta et al, 1996; Gorelick et al, 2005; Heinz et al, 2005). Detoxification from a short course of buprenorphine has been shown in a preliminary study to result in a significant increase in μ opioid [11C]-carfentanil binding in the inferofrontal cortex and anterior cingulate regions compared with controls (Zubieta et al, 2000). Therefore, it appears that similar increases in opioid receptor availability are seen during early abstinence from cocaine and alcohol, and preliminary data suggest a comparable increase in people with opioid dependence.

The evidence to date points to elevations in opioid binding being an acute effect of early abstinence, and our results in opioid dependence complement these findings. It is not clear whether these changes persist or even become additive with progressive detoxifications. In cocaine dependence, opioid receptor binding in some but not all regions returns to control levels within 1 week (Gorelick et al, 2005). In alcohol dependence the increase appears more persistent, with no reduction evident after 5 weeks of abstinence (Heinz et al, 2005). It was not possible to scan our participants after a period of abstinence owing to high relapse rates and strict residential rehabilitation programmes, but this would be valuable in future studies.

We found significant increases in [11C]-diprenorphine binding in the majority of regions analysed using the atlas, and significant increases in fusiform/parahippocampal gyri using exploratory voxel-based statistical parametric mapping, although increases were seen throughout the brain when the threshold for significance was lowered. It is not clear why an area incorporating the fusiform/parahippocampal gyri which is involved in processing visual associations and memory was highlighted by statistical parametric mapping. We found no difference in [11C]-diprenorphine binding between people with opioid dependence and controls in several of the a priori areas, notably the periaqueductal grey matter (in the brain-stem), nucleus accumbens and caudate. The template used for the brain-stem region is not precise enough to isolate the periaqueductal grey matter within the brain-stem region of interest, which may account for the lack of association there. However, we are surprised to find no association with the nucleus accumbens and caudate in the light of previous findings of increased receptor number in these areas during withdrawal from cocaine and alcohol. In the two studies of cocaine dependence, significant increases were seen in the ventral striatum and the anterior cingulate, frontal and temporal cortices, caudate and thalamus (Zubieta et al, 1996; Gorelick et al, 2005), whereas in alcohol dependence, significant increases were restricted to the ventral striatum (Heinz et al, 2005). In people with opioid dependence the changes were much more widespread, perhaps because of the direct pharmacological effect of opioids and possible changes in the endogenous opioid system.

**Opioid receptor availability and clinical variables**

We found no correlation between craving and opioid receptor availability, which is at variance with our hypothesis and previous findings in alcohol and cocaine dependence (Zubieta et al, 1996; Gorelick et al, 2005; Heinz et al, 2005). Our participants with opioid dependence demonstrated high scores on two rating scales for craving, which were comparable with those in a previous study (Weinstein et al, 1997) and with individuals maintained on methadone who had withdrawal induced by naloxone (Schuster et al, 1995) but were higher than scores for people maintained on methadone (Schuster et al, 1995). Furthermore, our participants experienced levels of and variance in craving scores that were comparable with earlier studies in which craving was induced and resulting brain activation detected (Daglish et al, 2001). Craving measures vary and so comparison with other studies is hampered. However, in our study we chose two commonly used scales with a total of seven craving subscales, so the absence of a correlation here is robust.

In other studies reporting a relationship between craving and opioid receptor levels, [11C]-carfentanil, a μ-selective tracer was used (Zubieta et al, 1996, 2000; Gorelick et al, 2005; Heinz et al, 2005). It may be that since [11C]-diprenorphine labels μ, κ and δ opioid receptors, μ receptor-related changes were obscured by alterations in the other subtypes. However we think this unlikely as the [11C]-diprenorphine signal correlated only with the reported μ opioid receptor density in each brain region and not with the κ and δ opioid receptor density. Nevertheless, it would be beneficial to repeat this study using a more selective opioid receptor tracer, such as [11C]-carfentanil, to determine whether the increase in opioid receptor binding demonstrated here is mainly due to increase in any particular subtype.

Opioid receptor binding levels were not related to withdrawal symptoms as found in cocaine and alcohol dependence (Zubieta et al, 1996; Gorelick et al, 2005). This is consistent with the clinical picture where opioid withdrawal can be ameliorated by non-opioid pharmacotherapy. We did not find a correlation between age and opioid receptor levels in either the group with opioid dependence or controls. In a [11C]-carfentanil PET study of healthy controls with a wider age range, increasing age was associated with higher opioid receptor levels in the neocortex (Zubieta et al, 1999). Our more limited age range and younger average age likely contributed to the absence of such a correlation. All of our group with dependence were tobacco smokers and controls were current non-smokers, but there was no correlation between quantity of cigarettes smoked and [11C]-diprenorphine binding. Furthermore, another study of alcoholism reported no significant interaction between smoking status and μ opioid receptor availability in patients and controls (Heinz et al, 2005).

**Limitations**

Although this study was appropriately powered to detect the measured effect in a PET study of this nature, it may have been underpowered to determine associations with clinical variables, especially craving. The studies reporting an association between craving and opioid receptor levels had dependent groups of 10, 17 and 25 respectively (Zubieta et al, 1996; Gorelick et al, 2005; Heinz et al, 2005). However, the participants in our study were craving at
similar levels and with a wide range of craving scores, making it likely that any association should have been apparent.

**Implications**

We have reported a significant widespread increase in brain opioid receptor availability in people with opioid dependence during early abstinence from methadone. Together with previous evidence, we argue that this reflects an increase after cessation of methadone rather than a chronic change. If this is the case, it could give us a crucial insight into the mechanisms that underlie opioid craving. Although clinically we know that substitution treatment is effective we do not know whether prolonged agonist exposure permanently alters brain neurochemistry and whether these changes hamper recovery. Furthermore, since such an increase in opioid receptors has also been shown in alcohol and cocaine dependence, this argues for a fundamental role of the opioid system in addiction, or at least in the early abstinence syndrome. The contribution of this to clinical states and treatment outcomes has yet to be fully characterised.

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