The role of opioids in restless legs syndrome: an $[11\text{C}]$diprenorphine PET study

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Summary

Opioids have been shown to provide symptomatic relief from dysaesthesias and motor symptoms in restless legs syndrome (RLS). However, the mechanisms by which endogenous opioids contribute to the pathophysiology of RLS remain unknown. We have studied opioid receptor availability in 15 patients with primary RLS and 12 age-matched healthy volunteers using PET and $[11\text{C}]$diprenorphine, a non-selective opioid receptor radioligand. Ligand binding was quantified by generating parametric images of volume of distribution ($V_d$) using a plasma-derived input function. Statistical parametric mapping (SPM) was used to localize mean group differences between patients and controls and to correlate ligand binding with clinical scores of disease severity.

There were no mean group differences in opioid receptor binding between patients and controls. However, we found regional negative correlations between ligand binding and RLS severity (international restless legs scale, IRLS) in areas serving the medial pain system (medial thalamus, amygdala, caudate nucleus, anterior cingulate gyrus, insular cortex and orbitofrontal cortex). Pain scores (affective component of the McGill Pain Questionnaire) correlated inversely with opioid receptor binding in orbitofrontal cortex and anterior cingulate gyrus. Our findings suggest that, the more severe the RLS, the greater the release of endogenous opioids within the medial pain system. We therefore discuss a possible role for opioids in the pathophysiology of RLS with respect to sensory and motor symptoms.

Keywords: PET; opiates; $[11\text{C}]$diprenorphine; pain; RLS

Abbreviations: IRLS = international restless legs scale; PLM = periodic limb movement; PLMS = periodic limb movement in sleep; RLS = restless legs syndrome; SPECT = single photon emission computed tomography; SPM = statistical parametric mapping; $V_d$ = volume of distribution


Introduction

Restless legs syndrome (RLS) is a common neurological disorder affecting up to 10% of the Caucasian population (Rothdach et al., 2000) and may lead to significant levels of morbidity. RLS exists in both primary (often hereditary) and secondary forms, and is clinically characterized by an urge to move associated with sensory, sometimes even painful sensations deep within the legs and feet. Symptoms occur in situations of rest and relaxation and are worse in the evening and at night. Voluntary movements provide temporary relief. RLS is a clinical diagnosis and criteria have been defined by the International RLS Study Group (Allen et al., 2003; Walters, 1995). In addition, involuntary leg movements, termed ‘periodic limb movements’ (PLM), may occur during sleep and lead to frequent arousal or awakening. The most severe problem for RLS patients is sleep disturbance and restlessness during the evening, which may impact on all other aspects of life.

The aetiology and pathophysiology of primary RLS remain unknown. Levodopa and dopamine agonists are symptomatically...
effective in the majority of RLS patients (Hening et al., 1999). However, imaging studies have failed to reveal any consistent functional changes in the nigrostriatal dopaminergic system, with several position emission tomography (PET) and single photon emission computed tomography (SPECT) studies showing either no (Trenkwalder et al., 1999; Eisensehr et al., 2001; Linke et al., 2004; Tribl et al., 2004) or only mild reductions in dopamine terminal [18F]dopa uptake, transporter binding, or postsynaptic dopamine D2 receptor binding (Turjanski et al., 1999; Ruottinen et al., 2000; Michaud et al., 2002; Mrowka et al., 2004). The resolution of PET and SPECT used in these studies was of the order of 5–8 mm and only examined striatal function and so substratal or brainstem/spinal changes in dopaminergic function would not have been detected.

Major components of RLS are dysaesthesias and pain, which appear to promote the urge to move. Consistent with this viewpoint, opioid receptor agonists, which are known to act predominantly on the pain system, have been shown to significantly improve RLS symptoms (Hening et al., 1986; Ondo, 2004; Trzepacz et al., 1984; Walters et al., 1993; Walters et al., 2001; review in: Walters, 2002).

Changes in opioid receptor availability in chronic pain syndromes such as rheumatoid arthritis and trigeminal neuralgia (Jones et al., 1999; Jones et al., 1991a) have previously been demonstrated using [11C]diprenorphine, a non-specific opioid receptor antagonist with similar affinities for mu, kappa and delta receptor subtypes, and PET. In these studies, ligand binding was reported to be decreased in areas involved in pain perception, including ‘prefrontal’, insular and cingulate cortices, thalamus and the basal ganglia, compatible with either heightened endogenous opioid release and/or receptor internalization. More recently, Willoch et al. (2004) investigated [11C]diprenorphine binding in central post-stroke pain in a limited number of patients and showed reduced binding in thalamus, parietal, secondary somatosensory, insular and lateral prefrontal cortices contralateral to the lesion as well as in anterior and posterior cingulate cortices along the midline and in midbrain grey matter. These findings were supported by Jones et al. who again measured [11C]diprenorphine binding in central neuropathic pain, main post-stroke pain, and found reductions in similar areas with exception of the secondary somatosensory cortex and prefrontal areas (Jones et al., 2004).

In addition to alterations in chronic pain, [11C]diprenorphine binding has been reported to be decreased in striatal regions in a number of hyperkinetic movement disorders including dyskinetic Parkinson’s disease (Piccini et al., 1997) and Huntington’s disease (Weeks et al., 1997). In the afore mentioned neurodegenerative diseases preclinical investigations have suggested that altered opioid transmission within the basal ganglia may, in part, be responsible for the genesis of involuntary movements (Augood et al., 1996; Henry et al., 1999; Henry et al., 2001).

We have investigated opioid receptor availability in patients with idiopathic RLS using [11C]diprenorphine PET and discuss a possible role for opioidergic dysfunction in the pathophysiology of this condition with respect to motor and pain symptoms. Statistical parametric mapping (SPM) was used to compare group means and to correlate opioid receptor binding with clinical ratings of RLS severity.

Methods

Subjects

Fifteen Caucasian patients with idiopathic RLS (five men; 10 women; mean age = 45.2, SD = 15.8 years) and 12 age-matched healthy volunteers (five men; seven women, mean age = 45.6, SD = 12.1; P = 0.95) were recruited from a specialized outpatient clinic at the Department of Clinical Neurophysiology, Goettingen, Germany, and by advertisements in London, UK, respectively.

All pre-examinations were performed at the Department of Clinical Neurophysiology in Goettingen, Germany, during a 2-day stay in hospital. A thorough medical and neurological examination was performed in all patients (TT and CT) and patients with severe medical or neurological disorders such as cardiovascular problems were excluded from participation in this study. All patients fulfilled the minimum clinical diagnostic criteria for RLS (Allen et al., 2003; Walters, 1995) and underwent two nights of polysomnography to confirm the diagnosis. Polysomnography provides an objective measure of PLM and their effect on sleep stages. The sleep profile of RLS patients characteristically consists of a reduced sleep efficiency caused by frequent awakenings mostly attributable to periodic limb movements in sleep (PLMS) and a low or absent percentage of deep sleep (stage 3 and 4). Using polysomnography the clinical diagnosis of RLS was confirmed and differential diagnoses such as respiratory disorders or parasomnias were ruled out. Polysomnographic recordings were analysed according to Rechtschaffen and Kales (1968) by an experienced sleep specialist (SH). Patients were included only if they had a PLMS-index of greater than five movements per hour or a severe sleep disorder consistent with the diagnosis of RLS. Patient 15 had no polysomnographic recordings owing to technical reasons and data from three patients (Patients 2, 11 and 13) were not valid enough to be correlated with clinical scores and PET data because of severe sleep disturbances (one of these had to be excluded from the SPM analysis anyway because of problems with blood collection during the PET scan, see further down). However, as these patients fulfilled the clinical diagnostic criteria and all had a positive family history of RLS, they were included in the study.

Periodic limb movements in sleep were counted visually and PLMS-indices per hour of total sleep time as well as the sleep efficiency were calculated (mean = 74.55%, SD = 14.42%, range = 52–94% for sleep efficiency; mean = 391.91 min, SD = 83.02 min, range = 281–569 min for total sleep time and mean = 36.31, SD = 33.62, range = 0–91.63 for PLMS/h).

Nerve conduction studies (electrophysiological tests) of the right peroneal and sural nerves were performed to exclude a peripheral neuropathy. Subjects also had blood tests (blood count, iron, ferritin, folic acid, vitamin B12, renal and thyroid function) to exclude iron deficiency, haematological or thyroid dysfunction. The above investigations were undertaken to rule out secondary forms of RLS.

RLS symptom severity was assessed using scores obtained on the International Restless Legs Scale (IRLS). This scale was developed and validated by the International Restless Legs Syndrome Study Group (IRLSSG, 2003) and contains questions...
that relate to the frequency and overall severity of (motor) symptoms (e.g. restlessness and urge to move), severity of sleep disturbances and the impact on the patient’s daily living. Using this scale, patients are asked to rate the above-mentioned features for the previous two weeks.

To assess pain severity, a German version of the McGill Pain Questionnaire (Stein and Mendl, 1988) was used. This questionnaire offers a list of 78 adjectives used to describe different qualities (sensory, affective and evaluative) and different degrees of pain severity. The words are divided into subclasses and rated from least to worst pain within each subclass (Melzack, 1975; Stein and Mendl, 1988). In contrast to the original version, where patients are asked to rate their current pain, we asked patients to select words that characterize their usual RLS symptoms, therefore these ratings do not refer to a limited time period.

Both scales were completed whilst patients were in hospital for pre-examinations.

Correlations of polysomnographic parameters (PLMS/h and sleep efficiency) with the individual scores of the IRLS and total- and sub-scores of the McGill Pain Questionnaire were calculated in SPSS® using a Pearson linear correlation.

Patients were either untreated or taking low doses of levodopa or dopamine agonists (Table 1). RLS medication was stopped at least 48 h prior to PET scanning. Neither patients nor controls had previously been treated with opioid agonists.

In London, UK, T1-weighted MRI scans were performed on the same day of PET scanning in all but one patient (Patient 15) in order to screen out any morphological abnormalities.

The study was approved by the Ethics Committees of the Georg-August University Goettingen, Germany, and the Hammersmith Hospitals Trust, London, UK. Written informed consent was requested separately by both review boards and was signed in Goettingen and in London. Permission to administer radioactivity was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC) of the Department of Health, UK. This study was performed according to the requirements of the Declaration of Helsinki.

### Scanning procedure

Positron emission tomography scans in 3D acquisition mode were performed at the Cyclotron Building, MRC Clinical Sciences Centre, Hammersmith Hospital, London, UK. Prior to administration of the radioactive tracer, a 5 min transmission scan was performed using a rotating point source of 150 MBq of $^{137}$Cs to correct acquired emission data for tissue attenuation. 185 MBq (5 mCi) of $[^{11}C]$diprenorphine in 5 ml of normal saline were injected intravenously as a bolus over 30 s and dynamic emission data were collected in list mode over the following 95 min using an ECAT EXACT3D PET scanner (model 966, CTI, Knoxville, TN, USA) (Spinks et al., 2000). Emission data were re-binned into 32 time frames, corrected for attenuation and scatter [using the model-based method of Watson et al. (1996)] and reconstructed using a reprojection algorithm (Kinahan and Rogers, 1989), with Colsher and ramp filters set at Nyquist frequency, into images with a spatial resolution of 5.1 mm × 5.1 mm × 5.1 mm (full width half maximum, FWHM). Arterial blood activity was sampled continuously over the whole time of the scan using a BGO (bismuth germanate) detector system (Ranicar et al., 1991) at pump rates of 300 ml/h (5 ml/min) for the first 10 min and of 150 ml/h (2.5 ml/min) thereafter. Discrete samples were taken at 5, 10, 20, 30, 40, 60, 75 and 90 min and processed for the determination of the ratio of radioactivity concentration in plasma and whole blood and the peripheral metabolism of $[^{11}C]$diprenorphine in order to create a metabolite corrected plasma input function.

### Quantification of $[^{11}C]$diprenorphine binding

$[^{11}C]$Diprenorphine uptake was quantified using spectral analysis with individual metabolite corrected plasma input

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### Table 1 Demographic data of RLS patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Age of onset (years)</th>
<th>Duration of disease (years)</th>
<th>Family history*</th>
<th>Medication** (per day)</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>24</td>
<td>14</td>
<td>10</td>
<td>+</td>
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<td>2</td>
<td>M</td>
<td>64</td>
<td>7</td>
<td>57</td>
<td>+</td>
<td>Pergolide 2 mg</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>62</td>
<td>47</td>
<td>15</td>
<td>+</td>
<td>Cabergoline 3 mg</td>
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<tr>
<td>4</td>
<td>F</td>
<td>47</td>
<td>42</td>
<td>5</td>
<td>–</td>
<td></td>
</tr>
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<td>5</td>
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<td>23</td>
<td>16</td>
<td>7</td>
<td>–</td>
<td>l-Dopa on demand</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>34</td>
<td>24</td>
<td>10</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>63</td>
<td>40</td>
<td>23</td>
<td>+</td>
<td>Pergolide 0.5 mg</td>
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<tr>
<td>8</td>
<td>M</td>
<td>62</td>
<td>26</td>
<td>36</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>49</td>
<td>35</td>
<td>14</td>
<td>+</td>
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<td>10</td>
<td>M</td>
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<td>23</td>
<td>20</td>
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<tr>
<td>11</td>
<td>F</td>
<td>53</td>
<td>23</td>
<td>30</td>
<td>+</td>
<td>l-Dopa 100 mg</td>
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<tr>
<td>12</td>
<td>F</td>
<td>30</td>
<td>28</td>
<td>2</td>
<td>+</td>
<td>l-dopa retard 100 mg</td>
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<tr>
<td>13</td>
<td>F</td>
<td>49</td>
<td>45</td>
<td>4</td>
<td>+</td>
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</tr>
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<tr>
<td>15</td>
<td>F</td>
<td>67</td>
<td>45</td>
<td>22</td>
<td>+</td>
<td>l-Dopa 100 mg</td>
</tr>
</tbody>
</table>

Mean ± SD 45.2 ± 15.8 29 ± 13.9 18.3 ± 14.5

*+, positive family history; –, negative family history; **all medication was stopped at least 48 h prior to PET scanning.
functions to create parametric images of ligand volume of distribution (Vd) (Cunningham and Jones, 1993). The Vd is the ratio of tissue to free plasma ligand concentration at equilibrium and provides an estimate of receptor binding (Jones et al., 1994). Parametric images of Vd were created using RPM (receptor parametric mapping, spectral analysis: MRCCU, Vin Cunningham and Roger Gunn) as implemented in Matlab5 (The MathWorks, Inc., Natick, MA, USA) and thus provided the Vd for each and every voxel (voxel size 2.096 mm × 2.096 mm × 2.43 mm). In one subject (Patient 11) the initial part of the on line blood collection was interrupted, rendering the plasma input function unreliable. This subject was, therefore, excluded from assessments of Vd. In order to include this subject in an analysis of study outcomes, parametric ratio images of specific to non-specific [11C]diprenorphine binding were created in addition to parametric images of Vd, using the occipital cortex as a reference region. By using a reference region approach, radioligand measurements in blood are no longer required to provide an input function. This dual approach of quantifying [11C]diprenorphine uptake using both spectral analysis and tissue specific:non-specific ratios has previously been employed by our laboratory (Piccini et al., 1997; Weeks et al., 1997). Ratio images were generated from 60 to 90 min following radioligand injection using software developed in house created in IDL (interactive data language, Research Systems International, Boulder, CO, USA).

Prior to statistical analysis, [11C]diprenorphine Vd and ratio images were normalized to the space defined by the Montreal Neurological Institute (MNI)/International Consortium for Brain Mapping (ICBM) T1-weighted 152 brain average as supplied with SPM99, using an [11C]diprenorphine template created in house from the PET scans of seven healthy volunteers. Smoothing was applied with a Gaussian kernel of 8 mm × 8 mm × 8 mm.

**Image analysis: statistical parametric mapping**

Statistical parametric mapping (SPM99) was applied to both parametric Vd and ratio images to localize mean group differences in [11C]diprenorphine uptake between RLS patients and controls on a voxel-by-voxel basis (Friston, 1995) (SPM99, Wellcome Department of Cognitive Neurology, London, UK). Within SPM, significant differences were assessed using parametric statistics. The resulting statistics had Student’s t distribution under the null hypothesis. Using SPM, values of the t statistic are corrected for multiple comparisons using a theory of random fields approach. They are converted into Z scores and assembled into an image (statistical parametric map). To visualize the statistical parametric maps, the threshold was set to P < 0.01 uncorrected excluding clusters with a spatial extent of < 50 voxels. Using the same significance threshold and cluster extent, comparisons to localize differences between patients and controls were performed using normalized ratio images. For both analyses, the threshold was reduced to P < 0.05 uncorrected with a cluster extent of 50 voxels when the first analysis did not reveal any significant results.

SPM was also used to localize clusters where ligand binding (using both Vd and ratio images) correlated significantly with individual scores of the IRLS and total- and sub scores (sensory, affective, evaluative, miscellaneous) of the McGill Pain Questionnaire. As described above, the threshold was initially set to P < 0.01 uncorrected with a cluster extent of 50 voxels and reduced to P < 0.05 uncorrected with a cluster extent of 50 voxels if no changes where localized at the P < 0.01 level. Therefore, by using the method described, we initially evaluated the entire brain volume (>200 000 voxels). However, clusters localized at the above stated thresholds were investigated further using regional volume correction. This was applied to the SPM map by using a single template object image which defined all of the regions for which we had an a priori hypothesis for change in opioid receptor availability in RLS. These hypotheses were based on previously reported activation (Firestone et al., 1996; Adler et al., 1997; Peyron et al., 2000; Casey, 2000a) and [11C]diprenorphine PET studies (Jones et al., 1991a; Jones et al., 1991b; Jones et al., 1999) in different conditions of clinical and experimental pain and an fMRI study investigating activated brain areas in RLS (Bucher et al., 1997). The regions included: caudate nucleus, putamen, thalamus, insular cortex, anterior cingulate gyrus, orbitofrontal cortex, amygdala, midbrain and pons. The primary sensory cortex was not included as the lateral (discriminating) pain system is relatively devoid of opioid receptors (see discussion). Using regional volume correction, voxels outside the above mentioned regions were not compared, therefore reducing the number of comparisons made and consequently the level of statistical correction required.

In a similar manner correlations between polysomnographic parameters (PLMS/h and sleep efficiency) and tracer binding were calculated.

Finally, we analysed the subgroup of untreated de novo patients (n = 9) in the same way as described above to test whether medication had a major influence on the results.

**Results**

**Patients**

Demographic details for the RLS patients are shown in Table 1. Clinical status, as assessed by IRLS scores and scores and sub scores of the McGill Pain Questionnaire, are shown in Table 2. The MRI scans performed in all patients (with the exception of Patient 15) were normal in every case.

Significant correlations of polysomnographic parameters (n = 11) were found between the IRLS and the sleep efficiency (r = −0.690; P = 0.019) and between the IRLS and the PLMS-index (r = 0.656; P = 0.028). Following a Bonferroni–Holm correction for multiple comparisons, none of these correlations remained significant.
Analysis of group means

Neither at a threshold of $P < 0.01$ uncorrected nor at the reduced threshold of $P < 0.05$ uncorrected (both times with a cluster extent of 50 voxels), did SPM localize any significant clusters of group mean decreased or increased opioid receptor binding between RLS patients and controls throughout the brain when assessing both $[11C]$diprenorphine $V_d$ and uptake ratio images. Furthermore, there were no correlations between $[11C]$diprenorphine $V_d$ or ratio images and age or disease duration. No gender differences in ligand binding were seen.

Correlation analysis: $V_d$ images

When applied to $V_d$ images, at a $P < 0.01$ uncorrected threshold and a cluster extent of 50 voxels, SPM localized negative correlations between $[11C]$diprenorphine binding and RLS symptom severity (IRLS score) ($n = 14$ patients) in bilateral insular, orbitofrontal and anterior cingulate cortices, and bilateral medial thalamus, amygdala and caudate nucleus (Fig. 1). High severity scores correlated with low opioid receptor availability. The voxel with the highest $t$ value was in the left amygdala ($t = 5.48, Z = 3.81$, SPM coordinates $-18, 8, -24$) and part of a large cluster of 2806 voxels ($P = 0.002$, corrected for the entire brain volume), which included the amygdala, insula and caudate nucleus in the left hemisphere and caudate nucleus and medial thalamus in the right hemisphere.

The results of the investigation examining clusters localized in the above analysis following regional volume correction are shown in Table 3. Regional cluster corrected $P$ values are quoted following regional volume correction, which was applied as outlined in the methods section.

In order to further examine the effect sizes of these regional negative correlations between $[11C]$diprenorphine $V_d$ and RLS severity (IRLS), individual regional $V_d$ data were extracted from 6 mm diameter spheres centred on the peak voxel of significant correlation within the amygdala, thalamus, anterior cingulate gyrus and orbitofrontal cortex. These correlations are shown in Fig. 2.

At the reduced threshold of $P < 0.05$ uncorrected with a cluster extent of 50 voxels, SPM also localized negative correlations between $[11C]$diprenorphine binding ($V_d$, $n = 14$) and the affective component of the McGill Pain Questionnaire bilaterally in orbitofrontal cortex, anterior cingulate gyrus and caudate nucleus (Fig. 3). The voxel with the highest $t$ value was in a large 2447 voxel cluster, which included the orbitofrontal cortex and right insula ($t = 4.19; Z = 3.23$; SPM coordinates: $16, 32, 16$; $P = 0.362$ (corrected for the entire brain)). After regional volume correction, applied as outlined above, none of the regional correlations remained significant.

Correlation analysis: ratio images

Interrogating images of specific:non-specific radioligand uptake ratios with SPM revealed no significant correlations when the significance threshold was set to $P < 0.01$ uncorrected with a cluster extent of 50 voxels. However, following reduction of the threshold to $P < 0.05$, the same regional pattern of negative correlations (amygdala, thalamus, caudate nucleus, anterior cingulate gyrus, insular and orbitofrontal cortex) was seen between $[11C]$diprenorphine specific:non-specific uptake ratios and the IRLS score when all RLS patients were included (Fig. 4). Regional volume correction yielded a significant cluster of 683 voxels centered on the right medial thalamus ($t = 2.93; Z = 2.52$; SPM coordinates: $6, -8, 12; P = 0.008$) and encompassing the medial thalamus and caudate nuclei bilaterally. For completeness, a ratio SPM analysis was also performed with subject 11 excluded.

Table 2 Clinical status of RLS patients assessed by IRLS and McGill Pain Questionnaire

<table>
<thead>
<tr>
<th>Patient</th>
<th>IRLS</th>
<th>McGill Pain Questionnaire</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sensory</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
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<td>17</td>
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<tr>
<td>15</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25.4 ± 6.9</td>
<td>12.9 ± 6.1</td>
</tr>
</tbody>
</table>
Negative correlations were also found between $^{[11]C}$diprenorphine ratio images ($n = 15$) and the affective component of the McGill Pain Questionnaire in the orbito-frontal cortex, left anterior cingulate gyrus and left caudate nucleus at $P < 0.05$ uncorrected threshold and a cluster extent of 50 voxels.

Table 3  Localized regional negative correlations between $^{[11]C}$diprenorphine $V_d$ ($n = 14$) and RLS severity (IRLS) following regional volume correction

<table>
<thead>
<tr>
<th>Region</th>
<th>SPM coordinates (mm)</th>
<th>Cluster size (voxels)</th>
<th>$t$ Score</th>
<th>$Z$ Score</th>
<th>Regional volume-corrected $P$ value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$x$</td>
<td>$y$</td>
<td>$z$</td>
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</tr>
<tr>
<td>Right caudate</td>
<td>8</td>
<td>18</td>
<td>2</td>
<td>193</td>
<td>4.53</td>
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<tr>
<td>Left caudate</td>
<td>$-6$</td>
<td>12</td>
<td>2</td>
<td>270</td>
<td>3.90</td>
</tr>
<tr>
<td>Medial thalamus*</td>
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<td>$-4$</td>
<td>4</td>
<td>269</td>
<td>3.90</td>
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<td>Anterior cingulate gyrus*</td>
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<tr>
<td>Left insula</td>
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<td>$-4$</td>
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<td>376</td>
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<td>Orbitofrontal cortex</td>
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<td>64</td>
<td>$-18$</td>
<td>63</td>
<td>3.52</td>
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<tr>
<td>Right amygdala</td>
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<td>$-20$</td>
<td>119</td>
<td>3.47</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>$-18$</td>
<td>8</td>
<td>$-24$</td>
<td>138</td>
<td>5.48</td>
</tr>
</tbody>
</table>

N.S. = not significant; *Cluster extends over both left and right sides.

(i.e. $n = 14$: the same population as for the $V_d$ analysis) and the same results as for the above mentioned $n = 15$ ratio analysis were obtained.

Negative correlations were also found between $^{[11]C}$diprenorphine ratio images ($n = 15$) and the affective component of the McGill Pain Questionnaire in the orbito-frontal cortex, left anterior cingulate gyrus and left caudate nucleus at $P < 0.05$ uncorrected threshold and a cluster extent of 50 voxels.

No clusters of positive correlation between $^{[11]C}$diprenorphine $V_d$ or ratio images and either the IRLS scores or the affective component of the McGill Pain Questionnaire were localized. Neither the total score nor other components of the McGill Pain Questionnaire correlated with ligand binding.

The correlation analysis of sleep laboratory measurements and $^{[11]C}$diprenorphine binding ($n = 11$) showed a significant positive correlation in two clusters ($P < 0.01$), one included...
parts of pre- and postcentral gyrus (representing neck, arm, shoulder, very close to the edge of the image); the other was within the inferior posterior temporal lobe.

There was no negative correlation between tracer binding and PLMS/h at significance thresholds up to $P < 0.05$. Furthermore, there were no significant correlations with the sleep efficiency.

Correlation analyses using only the subgroup of untreated de novo patients ($n = 9$) showed the same regional patterns of negative correlation between $[^{11}C]$diprenorphine and IRLS score as described above, although these correlations failed to reach significance.

**Discussion**

This is the first study to measure opioid receptor binding in RLS patients using PET. We have found significant negative correlations between opioid receptor availability and severity of RLS symptoms in brain regions involved in the medial affective pain system. Using both an $[^{11}C]$diprenorphine $V_d$ and specific:non-specific uptake ratio approach to ligand quantification, negative correlations were seen in orbitofrontal, insular and cingulate cortices, medial thalamus, caudate nucleus and amygdala bilaterally.

This decrease in $[^{11}C]$diprenorphine binding may indicate increased occupancy of opioid receptors by endogenous opioids and, therefore, reflect their heightened release. Thus, one possible interpretation is that the more severe the symptoms of RLS the greater the endogenous release of opioids in the medial affective pain system. Furthermore, scores of the affective component of the McGill Pain Questionnaire were inversely correlated with ligand binding in orbitofrontal areas and anterior cingulate gyrus. Again, this may indicate increased opioid release caused by pain/dysaesthesia leading to decreased opioid receptor availability. Other possible explanations for reduced $[^{11}C]$diprenorphine binding, such as receptor internalization and/or receptor down
regulation cannot be distinguished from the above-mentioned hypothesis with the technique of PET, but seem to be less likely. Atrophy of specific brain regions as a possible explanation for reduced ligand binding is ruled out by normal MRI scans.

\[11C\]Diprenorphine PET did not reveal any regional differences in opioid receptor availability when categorically comparing the patient and control group means.

We have investigated opioid receptor availability using \([11C]\)diprenorphine PET in a homogeneous group of RLS patients as pre-examinations excluded all patients with secondary forms of RLS and 13 out of 15 patients reported a positive family history suggesting mostly hereditary forms. All patients fulfilled the criteria for a chronic syndrome with a moderate to severe manifestation of symptoms over more than six months. Scores of the IRLS were evenly distributed and the lowest score was 15, which is the minimum value now required for inclusion in many treatment trials. Not all of our RLS patients were untreated \textit{de novo} patients: three were taking low doses of \(L\)-dopa formulations and three were receiving dopamine agonists (medication was stopped 48 h prior to PET). This medication could have affected our PET results; however, this seems to be unlikely given the fact that we found similar patterns of reduced \([11C]\)diprenorphine binding as for the whole patient group when correlating tracer binding and RLS/pain severity in the subgroup of nine untreated \textit{de novo} patients. These correlations did not reach significance due to reduced statistical power in this smaller subset.

The sleep efficiency as well as the PLMS-index was correlated with RLS severity as measured with the IRLS. This supports findings by Garcia-Borreguero and colleagues, who recently reported significant correlations between IRLS scores and various sleep laboratory measurements (Garcia-Borreguero et al., 2004). However, there is some doubt about the correlation with PLMS/h as the individual values were not evenly distributed.

As the correlation of PET data with the PLMS-index is positive in contrast to negative correlations with the severity scale and occurs in anatomical regions that have not been shown to be activated in RLS/during periodic leg movements (Bucher et al., 1997) we do not believe that they represent true biological findings but occurred by chance and/or due to edge effects.

Although opioids are known to reduce sensory and motor symptoms in RLS patients, the involvement of pain systems in the pathophysiology of RLS has previously only been demonstrated using \(H_2[15O]\) PET, as evidenced by changes in regional cerebral blood flow (rCBF) in two RLS patients (San Pedro et al., 1998). In these patients (father and daughter), rCBF was significantly decreased in caudate nucleus and significantly increased in the thalamus bilaterally with
increasing pain. Levodopa reduced pain and normalized blood flow in these two cases. Using functional magnetic resonance imaging (fMRI), Bucher et al. (1997) showed activation in the cerebellum bilaterally and in the thalamus contralaterally to the affected leg during the condition of sensory leg discomfort. More recently, increased ratings of pin-prick pain were reported in untreated RLS patients indicating static hyperalgesia that was more pronounced in the lower limb and reversed by long-term dopaminergic treatment (Stiasny-Kolster et al., 2004). In addition to these findings we report alterations in opioid receptor availability in structures that constitute the medial pain system in a large group of idiopathic RLS patients.

Pain perception can be divided into sensory-discriminative and affective-motivational components. Post-mortem studies (Pfeiffer et al., 1982; Atweh and Kuhar, 1983; Peckys and Landwehrmeyer, 1999) as well as functional imaging studies using \([11C]diprenorphine PET (Jones et al., 1999)\) have shown high levels of opioid receptor binding in structures known as the medial pain system. This system projects through medial and intralaminar nuclei of the thalamus to several cortical and limbic regions: frontal and insular cortices and anterior cingulate gyrus. It is thought to mediate affective-motivational aspects of pain such as emotional reactions, arousal and attention to the stimulus, as well as the drive to escape from the noxious stimuli (Treede et al., 1999). In contrast to the medial (affective) pain system, the lateral (sensory-discriminative) pain system (projecting to the primary sensory cortex) is relatively devoid of opioid receptors (Jones et al., 1991b).

Following experimentally induced pain in the masseter muscles, significant negative correlations between mu-opioid receptor binding measured with \([11C]carfentanil PET and affective subscores of the McGill Pain Questionnaire have previously been shown bilaterally in the dorsal anterior cingulate cortex and thalamus and ipsilaterally in the nucleus accumbens. Additional correlations with McGill Pain Questionnaire sensory scores were found in thalamus, nucleus accumbens and amygdala ipsilateral to the painful stimulus (Zubieta et al., 2001).

In contrast to these findings, we found no correlations in the nucleus accumbens, possibly due to the fact that we used a non-specific opioid receptor antagonist as a PET-radioligand, which binds similarly to all three subtypes of opioid receptors. However, even more important may be the fact that our RLS patients were not experiencing frank pain during the scan. In contrast to the study of Zubieta et al. (2001) who studied acute, experimentally induced pain, the dyssynergia in RLS is a chronic condition and static mechanical hyperalgesia has been shown to occur in RLS patients indicating permanent changes in pain modulation mechanisms (Stiasny-Kolster et al., 2004); therefore, opioid binding changes may differ from those changes that occur during acute pain. Furthermore, we found negative correlations of \([11C]diprenorphine binding in the medial pain system not only with the McGill Pain Questionnaire but also with IRLS scores, a clinical score assessing RLS severity, which is biased towards motor (restlessness) symptoms rather than sensory (pain) phenomena. There were no correlations with the sensory part of the McGill Pain Questionnaire, which is explicable if one considers the paucity of opioid receptors in the lateral pain system, which is responsible for mediating sensory-discriminative aspects of pain perception (Jones et al., 1991b).

The cluster localized in the cerebellum (Fig. 3) in the correlations between \([11C]diprenorphine V_d and the affective component of the McGill Pain Questionnaire was not seen when correlating this clinical score with ratio images and given the predominantly white matter and mid line location of this cluster we cannot rule out this being an artefact. However, the above mentioned fMRI study by Bucher et al. (1997) has shown activation in the cerebellum during sensory RLS symptoms and the presence of opioid receptors in this brain region was proven by \([11C]diprenorphine PET, mRNA expression and autoradiography studies (Schadrack et al., 1999). Furthermore, changes in rCBF during experimentally induced pain and following the administration of opioid receptor agonists have also been shown in the cerebellum (Firestone et al., 1996; Peyron et al., 2000; Casey et al., 2000b).

Although our negative correlations occurred in regions serving the medial pain system, only a minority of our RLS patients described pain as a major symptom. In personal interviews several patients reported their symptoms to be ‘painful in some way, but not like a typical pain such as toothache’ and finally judged these feelings as being ‘non-painful’. The McGill Pain Questionnaire offers a list of descriptions and patients were asked to choose those words that described their symptoms best. Therefore, we obtained an impression of the quality and quantity of the patients’ usual RLS symptoms and found that by a standardized questionnaire symptoms were rated as being painful although the quality of this pain seemed to be somewhat different from ‘typical pain’ as indicated by the discrepancy between subjective statements and standardized scores. Mean values of McGill Pain Questionnaire total- and subscores in RLS (Table 2: mean = 23.3, SD = 9.6 for the total score) were within the middle range compared to other chronic pain syndromes [e.g. arthritis: McGill Pain Questionnaire total score of 18.8, back pain: total score of 26.3, (Melzack, 1975)].

We found correlations between opioid receptor binding and severity scores not only in areas of the medial pain system but also in the caudate nucleus and amygdala bilaterally. The medial pain system as well as the amygdala is known to interact with the basal ganglia which Chudler and Dong (1995) speculated might play a role in the integration of incoming sensory information, so aiding planning of a coordinated motor response to pain perception. As the basal ganglia receive information from areas involved in sensory-discriminative as well as affective-motivational processing of painful stimuli this may include both direction
and speed of escape behaviour and the motivational drive to terminate the noxious stimulus.

It is possible that such basal ganglia motor activity following noxious stimulation might be regulated via dopamine–opioid interactions. For example, Chudler and Dong (1995) stated that reductions in central levels of dopamine are able to reverse the analgesia caused by opioids. Apomorphine has been shown to have a biphasic effect on morphine induced analgesia with lower doses attenuating analgesia (probably via presynaptic autoreceptor stimulation) and higher doses potentiating the antinociceptive effect of morphine (via postsynaptic dopamine receptor stimulation) (Gupta et al., 1989; Paalzow and Paalzow, 1983). Furthermore, dopamine–opiate interactions seem to depend on the type of the stimulus as well as on the response/response selection mechanisms evoked by this stimulus (Dennis and Melzack, 1983) and on the brain area integrating the response (Gupta et al., 1989; Paalzow and Paalzow, 1983). With respect to striatal and extrastriatal regions, opioid receptor agonists increase D2 and D3 receptor binding of PET radioligands [$^{11}$C]raclopride for striatal (Hagelberg et al., 2002) and [$^{11}$C]FLB 457 for extrastriatal dopamine receptor binding (Hagelberg et al., 2004) and decrease binding of the SPECT tracer [$^{123}$I]beta-CIT to presynaptic dopamine transporters (Bergstrom et al., 1998). This may reflect reduced dopamine release and increased dopamine reuptake, but is dependent on the kind of the opioid receptor agonist (Lubetzki et al., 1982). Whether and how these different mechanisms contribute to the pathophysiology of RLS remains unclear at this time; dopamine–opiate interactions are very complex, and may occur also on a spinal level [for review see Trenkwalder and Paulus (2004)].

We speculate that motor symptoms and especially the restlessness in RLS result from a disturbed balance of dopamine–opiate inputs to brain regions involved in motor actions and/or pain perception and may represent an aberrant behavioural response to sensory input. This might also explain why both dopaminergic agents and opioids are almost equally effective in RLS treatment. Furthermore, an altered balance between dopamine and opioid action rather than an absolute deficit of one neurotransmitter might be a reason for our failing to demonstrate mean group differences between patients and controls in opioid receptor availability as well as for the inconsistent findings of other imaging studies investigating dopaminergic function in the basal ganglia. Discussing their findings, authors of these studies raised the possibility of changes in dopaminergic systems other than the nigrostriatal projection, for example spinal and the diencephalic dopaminergic system thought to be involved in pain regulation (Lindvall et al., 1983).

In contrast to other hyperkinetic movement disorders where changes in opioid receptor availability have been shown in the basal ganglia, for example reduced ligand binding in striatal regions in dyskinetic Parkinson’s disease (Piccini et al., 1997) and decreased [$^{11}$C]diprenorphine uptake in caudate nucleus and putamen in patients with Huntington’s disease (Weeks et al., 1997), in patients with idiopathic RLS opioid receptor function seems to be affected primarily in sensory and association but not in motor areas. This might suggest hyperkinetic motor symptoms in RLS are secondary to sensory symptoms. Consistent with this view is that sensory discomfort and the urge to move are the first symptoms in RLS followed by a voluntary or involuntary (PLM) motor response (Pelletier et al., 1992; Trenkwalder et al., 1996).

Our finding that mean [$^{11}$C]diprenorphine binding was not different between the patient and control group may indicate that there is not an overall change of endogenous opioid transmission in RLS. Increased and possibly abnormal sensory input might cause a secondary deficit of endogenous opioids that results in insufficient levels of endogenous opioids. The source of these abnormal sensations in RLS still remains unknown. The primary cause of RLS may lie distal to supraspinal structures. In support of this view, several groups have reported signs of subclinical polyneuropathies in idiopathic RLS patients (Iannaccone et al., 1995; Rutkove et al., 1996; Polydefkis et al., 2000), abnormal cutaneous thermal thresholds indicating small fibre neuropathy (Happe and Zeitlof, 2003; Schattschneider et al., 2004) and the occurrence of RLS following spinal cord injury (Hartmann et al., 1999; Lee et al., 1996). This hypothesis could not be addressed further in this study.

Summarizing our findings, we have been able, for the first time, to demonstrate a central nervous system involvement of opioids in the pathophysiology of RLS. Furthermore, we have shown that pain is an underlying problem in RLS patients and suggested that motor symptoms in RLS are secondary to sensory symptoms. Derangement of opioid binding in RLS provides a rationale for using opioids in RLS treatment.

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