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# Thermal hypoaesthesia differentiates secondary restless legs syndrome associated with small fibre neuropathy from primary restless legs syndrome

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**This study aimed to assess thermal and mechanical perception and pain thresholds in primary idiopathic restless legs syndrome and secondary restless legs syndrome associated with small fibre neuropathy. Twenty-one patients (age: 53.4 ± 8.4, $n = 3$, male) with primary restless legs syndrome and 13 patients (age: 63.0 ± 8.2, $n = 1$, male) with secondary restless legs syndrome associated with small fibre neuropathy were compared with 20 healthy subjects (age: 58.0 ± 7.0; $n = 2$, male). Differential diagnosis of secondary restless legs syndrome associated with small fibre neuropathy was based on clinical symptoms and confirmed with skin biopsies in all patients. A comprehensive quantitative sensory testing protocol encompassing thermal and mechanical detection and pain thresholds, as devised by the German Research Network on Neuropathic Pain, was performed on the clinically more affected foot between 2 pm and 1 am when restless legs syndrome symptoms were present in all patients. Patients with primary restless legs syndrome showed hyperalgesia to blunt pressure ($P < 0.001$), pinprick ($P < 0.001$) and vibratory hyperaesthesia ($P < 0.001$). Patients with secondary restless legs syndrome associated with small fibre neuropathy showed thermal hypoaesthesia to cold (Aδ-fibre mediated) and warm (C-fibre mediated) (all $P < 0.001$) and hyperalgesia to pinprick ($P < 0.001$). Static mechanical hyperalgesia in primary and secondary restless legs syndrome is consistent with the concept of central disinhibition of nociceptive pathways, which might be induced by conditioning afferent input from damaged small fibre neurons in secondary restless legs syndrome.**

**Keywords:** quantitative sensory testing; restless legs syndrome; small fibre neuropathy; skin biopsy
**Abbreviations:** RLS = restless legs syndrome

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# Introduction

Even though population studies have shown that idiopathic restless legs syndrome (RLS) is one of the most frequent neurological disorders with a prevalence of 6–12% in Western countries (Berger and Kurth, 2007), a coherent aetiological concept incorporating all facets of RLS pathophysiology has not yet been established. Diagnosis of RLS is based on four standardized minimal obligatory criteria introduced and revised by the International Restless Legs Study Group (Walters 1995; Allen *et al*., 2003). In addition, a previous study by Stiasny-Kolster *et al.* (2004) demonstrated that patients with idiopathic RLS have a static mechanical hyperalgesia to pinprick stimuli, pointing to a central sensitization or disinhibition of Aδ-fibre-mediated pinprick perception. However, RLS symptoms are not restricted to the idiopathic form but may also develop as part of other clinical syndromes within the context of peripheral neuropathies involving small fibre lesion. Small fibre neuropathies can be categorized as peripheral neuropathies including damage to small calibre C- and Aδ-afferent neurons (Sumner *et al.,* 2003; Hoitsma *et al.,* 2004; ørstavik *et al.,* 2006; Laaksonen *et al.,* 2008; Obermann *et al.,* 2008). The performance of small diameter myelinated (Aδ) and unmyelinated (C) fibres is reduced in small fibre neuropathy, thus affecting their role in autonomic function (Stewart *et al.,* 1992), temperature and pain sensation (Devigili *et al.*, 2008). By definition, large fibre performance is intact (Devigili *et al.,* 2008; Scherens *et al.,* 2009). Nerve-conduction studies and electromyography, both of which are daily routine neurophysiological methods, assess large fibre function and are therefore normal in small fibre neuropathy. Accordingly, clinical examination of patients with small fibre neuropathy often reveals only discrete abnormalities specific for small fibre-mediated sensory modalities, e.g. temperature-perception deficits. Some of the more overt clinical features of patients with small fibre neuropathy are burning or shooting pain, paraesthesias and numbness (Hoitsma *et al.,* 2004; Ho *et al.,* 2009). Symptoms are usually distally accentuated and length dependent (Holland *et al.,* 1997; Devigili *et al.,* 2008). Small fibre-neuropathy diagnosis can be confirmed invasively with a skin biopsy, which shows a length-dependent loss of epidermal nerve-fibre density (Holland *et al.,* 1997). In contrast, quantitative sensory testing is a non-invasive psychophysical test assessing the function of small and large sensory nerve fibres, considered a 'potentially useful tool' for the measurement of sensory impairment according to the American Academy of Neurology (Shy *et al.,* 2003). Our study aimed to assess the complete somatosensory phenotype in idiopathic versus secondary RLS. We addressed the following questions: (i) are there distinct somatosensory profiles in idiopathic versus secondary RLS? and (ii) if so, are these signs suggestive of possible underlying neurobiological mechanisms such as central disinhibition, sensitization or peripheral nerve-fibre damage?

# Methods

All subjects and patients were able to understand the instructions of the quantitative sensory testing protocol. All participants gave their written consent. The study was performed in accordance with guidelines for good clinical practice and the Declaration of Helsinki and approved by the ethics committee of the Georg August University in Goettingen, Germany.

## Patients

We included 34 consecutive ordinary patients from the walk-in movement-disorders clinic at the Department of Clinical Neurophysiology of the Georg August University in Goettingen. Twenty-one patients (age: 53.4 ± 8.4 years, three male and 18 female) with primary idiopathic RLS and 13 patients (age: 63.0 ± 8.2 years, one male and 12 female) with secondary RLS due to small fibre neuropathy were compared with published quantitative sensory testing reference data of the German Research Network on Neuropathic pain, compensating for age and gender effects. In a second classical approach, patient data were compared with 20 healthy subjects (age: 58 ± 7.0 years; range: 46–75 years; two male and 18 female). The mean age of this control group was between both of the patient groups. All control subjects were free of any medication affecting quantitative sensory testing data, or any relevant neurological or pain disease, such as back pain or the presence of migraine, during the last 6 months prior to investigation. The diagnosis of RLS was made in accordance with the four revised essential criteria, as published by the International Restless Legs Syndrome Study Group (Walters 1995; Allen *et al.,* 2003). Peripheral sensory and motor nerve-conduction velocities (sural and peroneal or tibial nerve) were normal in patients and controls. Differential diagnosis of small fibre neuropathy and secondary RLS was initially made based on clinical findings (Table 1). Skin biopsy confirmed the clinical diagnosis of small fibre neuropathy in all patients with secondary RLS associated with small fibre neuropathy.

## Experimental design

A comprehensive quantitative sensory testing protocol encompassing thermal and mechanical detection and pain thresholds, as devised by the German Research Network on Neuropathic Pain, was performed on the dorsum of the clinically more affected foot between 2 pm and 1 am, i.e. during the symptomatic phase of RLS in each patient (Rolke *et al.,* 2006*a*, *b*). RLS medication was paused at least 24 h or five drug half-lives prior to quantitative sensory testing.

## Quantitative sensory testing protocol

The quantitative sensory testing protocol of the German Research Network on Neuropathic Pain consisted of seven tests measuring 13 parameters (Rolke *et al*., 2006*a*, *b*), including thermal and mechanical stimuli, specifically thermal detection thresholds for cold, warm and paradoxical heat sensations during alternating warm and cold stimuli (thermal sensory limen procedure), as well as cold and heat pain thresholds. Thermal thresholds were tested using a thermal sensory analyser (TSA 2001-II; Medoc, Israel; Yarnitsky and Sprecher, 1994). Mechanical detection thresholds were analysed using von Frey hairs (Optihair2-Set, Marstock Nervtest, Germany; Fruhstorfer *et al*., 2001). Mechanical pain thresholds, mechanical pain sensitivity, and the wind-up ratio as the perceptual correlate of temporal pain summation were assessed using a set of custom-made pinprick stimulators (Magerl *et al*., 1998; Baumgärtner *et al*., 2002). Dynamic mechanical allodynia was assessed with a set of light tactile stimulators that were applied in a balanced order intermingled with the pinprick

**Table 1 Demographic data of patients with primary and secondary RLS**

| Patient no. | Gender | Age | IRLSS | Age at onset | Duration (year) | Haemoglobin A1c | Family history | Sensory symptoms | Medication |
|---|---|---|---|---|---|---|---|---|---|
| Primary RLS | | | | | | | | | |
| 1 | F | 61 | 31 | 18 | 43 | 5.3 | No | Tingling paraesthesias | Rotigotine |
| 2 | F | 61 | 15 | 25 | 36 | 6.2 | Yes | Electrifying dysaesthesia, shooting pain | Ropinirole |
| 3 | M | 42 | 38 | 24 | 18 | 5.7 | Yes | Tingling paraesthesias | Tilidine/pregabalin |
| 4 | F | 58 | 28 | 49 | 9 | n.a. | No | Tingling paraesthesias | *de novo* |
| 5 | F | 51 | 23 | 47 | 4 | 5.8 | No | Tingling paraesthesias | None |
| 6 | F | 55 | 35 | 48 | 7 | 6.0 | Yes | Tingling paraesthesias | Pramipexole/tilidine |
| 7 | F | 55 | 34 | 53 | 2 | 6.1 | Yes | Tingling paraesthesias | Ropinirole |
| 8 | F | 43 | 26 | 35 | 8 | 5.7 | No | Tingling paraesthesias | None |
| 9 | F | 67 | 37 | 57 | 10 | 6.5 | Yes | Dragging paraesthesias | Pramipexole/ L-DOPA |
| 10 | F | 62 | 33 | 47 | 15 | 5.3 | Yes | Dragging paraesthesias | Ropinirole/tilidine |
| 11 | F | 66 | 27 | 51 | 15 | 5.5 | No | Tingling paraesthesias | Pramipexole |
| 12 | F | 46 | 21 | 45 | 1 | 5.6 | No | Dragging paraesthesias | None |
| 13 | F | 43 | 37 | 29 | 14 | 6.3 | No | Itching paraesthesias | Pramipexole/tilidine |
| 14 | F | 59 | 26 | 50 | 9 | 6.0 | Yes | Dragging dysaesthesias | Rasagiline |
| 15 | F | 48 | 39 | 47 | 1 | 5.8 | Yes | Sensation of tension | Ropinirole |
| 16 | M | 36 | 31 | 35 | 1 | n.a. | No | Tingling paraesthesias | *de novo* |
| 17 | F | 58 | 29 | 45 | 13 | 5.6 | Yes | Dragging and tingling paraesthesias | Rotigotine/pregabalin |
| 18 | F | 51 | 35 | 16 | 35 | 6.1 | Yes | Only urge to move | Pramipexole |
| 19 | M | 54 | 22 | 51 | 3 | 6.6 | Yes | Sensation of pressure | Rasagiline/L-DOPA |
| 20 | F | 58 | 29 | 45 | 13 | 5.7 | Yes | Tingling paraesthesias | Pramipexole/tilidine |
| 21 | F | 47 | 28 | 44 | 3 | 6.1 | Yes | Tingling paraesthesias | Tilidine |
| Mean | | 53.4 | 29.7 | 41.0 | 12.4 | 5.9 | | | |
| Secondary RLS | | | | | | | | | |
| 1 | F | 60 | 22 | 51 | 9 | 6.3 | Yes | Dragging pain | Pregabalin/ropinirole |
| 2 | F | 57 | 22 | 45 | 12 | 5.9 | Yes | Dragging pain | Pregabalin/ropinirole |
| 3 | F | 66 | 30 | 31 | 35 | 6.6 | Yes | Painful cramps | Pregabalin/tilidine |
| 4 | F | 63 | 29 | 57 | 7 | 6.0 | No | Shooting and dragging pain, cramps | Tilidine/ropinirole |
| 5 | M | 69 | 36 | 59 | 10 | 5.6 | No | Tingling and painful Dysaethesias | Pregabalin/tilidine/rotigotine |
| 6 | F | 64 | 29 | 44 | 20 | 6.4 | Yes | Burning pain | Responsive to gabapentin |
| 7 | F | 72 | 35 | 53 | 19 | 7.9 | No | Burning pain | Pregabalin |
| 8 | F | 61 | 14 | 59 | 2 | 5.9 | Yes | Numbness | *de novo* |
| 9 | F | 71 | 25 | 34 | 37 | 6.2 | No | Dragging pain, cramps | L-DOPA, pregabalin stopped |
| 10 | F | 67 | 30 | 48 | 19 | 6.5 | No | Unspecific pain | Cabergoline, pregabalin stopped |
| 11 | F | 73 | 23 | 37 | 36 | 6.4 | No | Burning pain | Ropinirole/tilidine |
| 12 | F | 47 | 29 | 43 | 4 | 6.0 | No | Burning pain | Pregabalin |
| 13 | F | 49 | 26 | 46 | 3 | 5.8 | No | Prickling pain, numbness | Pregabalin |
| Mean | | 63.0 | 26.9 | 46.7 | 16.4 | 6.3 | | | |
| *t*-test (*P*-value) | | <0.01 | 0.21 | 0.15 | 0.36 | <0.05 | | | |

Patients' parameters were compared with unpaired *t*-tests.
IRLSS = International Restless Legs Syndrome Study; n.a. = not applicable.

stimuli. Subjects rated pain sensations on a 0–100 numerical scale for the tactile and pinprick stimuli to calculate an s/r-function for mechanical pain sensitivity. Vibration-detection thresholds were determined as a disappearance threshold using a Rydel–Seiffer graded tuning fork (64 Hz, 8/8 scale). To determine the pressure-pain threshold, we employed a series of increasing ramps with a pressure-gauge device (Wagner Instruments, USA) exerting a pressure up to 20 kg/cm$^2$ or approximately 2000 kPa (Kosek *et al*., 1999) with a probe-contact area of 1 cm$^2$ over muscle tissue of the medial aspects of the foot (instep muscle). The quantitative sensory testing protocol has previously been shown to be valid, reliable (Geber *et al*., 2007) and void of left-to-right side difference (Rolke *et al*., 2006*a*, *b*).

## Statistical analysis

Cold-pain, heat-pain and vibration-detection thresholds were normally distributed as analysed by the Kolmogorov–Smirnov test. Paradoxical heat sensations were analysed with the Mann–Whitney U-test. All other parameters were normally distributed in log-space and were transformed logarithmically before statistical analysis (Rolke *et al.*, 2006*a*). All statistical calculations were performed using 'Statistica' software for Windows (StatSoft Inc., USA). Quantitative sensory testing values were *z*-transformed using the expression:

$$Z\text{-score} = (\text{mean}_{\text{individual RLS patient}} - \text{mean}_{\text{controls}})/\text{SD}_{\text{controls}}$$

The *Z*-score quantitative sensory testing data of each patient were compared with the group means of the healthy controls. *Z*-scores above '0' indicate a gain of function when the patient is more sensitive to the tested stimuli compared with controls, while *Z*-scores below '0' indicate a loss of function referring to a lower sensitivity of the patient. Two different control groups were used for comparing the patients' quantitative sensory testing data. Twenty healthy control subjects were investigated with a mean age between both patient groups. In this classical approach, differences between the groups were tested with one-way analysis of variance (ANOVA). *Post hoc* group comparisons were calculated using least significant differences tests. However, to fully compensate for age effects, single patient data were *z*-transformed according to quantitative sensory testing reference data from the German Research Network on Neuropathic Pain. Using this approach, quantitative sensory testing data from each patient were analysed, controlled for age and gender effects and matched to a larger set of healthy controls of the same age decade. For every quantitative sensory testing value, we used paired *t*-tests versus O (Bonferroni-corrected for multiple comparisons) to test if it was significantly different from the mean of controls (represented by a *Z*-score of 0; Fig. 1). All *Z*-score quantitative sensory testing data are presented as means ± SEM. Patients' parameters were compared with unpaired *t*-tests.

## Skin biopsy

Skin biopsies were performed with a disposable 4-mm punch at the distal leg (10 cm above the lateral malleolus) or the dorsum pedis, using a sterile technique and lidocaine injection for local anaesthesia.

Skin tissue was immediately immersed in 2% paraformaldehyde–lysine–periodate-fixative, transferred to cryoprotectant solution (20% glycerole in phosphate buffer) after 24 h, and stored at −80°C. Then, 40-μm cryostat sections were immunostained with mouse anti-protein gene product (PGP) 9.5 antibody (NCL-L-PGP9.5, Novocastra, Newcastle upon Tyne, UK), 1:20 dilution, using a free-floating technique and Cy2-coupled anti-mouse secondary antibodies (http://www.dianova.de). At least seven sections were evaluated per patient, corresponding to a median of 29.6 mm (min: 19.5 mm; max: 36.5 mm) of epidermal length. Microscopic evaluation was performed using an Olympus light/fluorescence BX51 microscope (Olympus Optical Co.

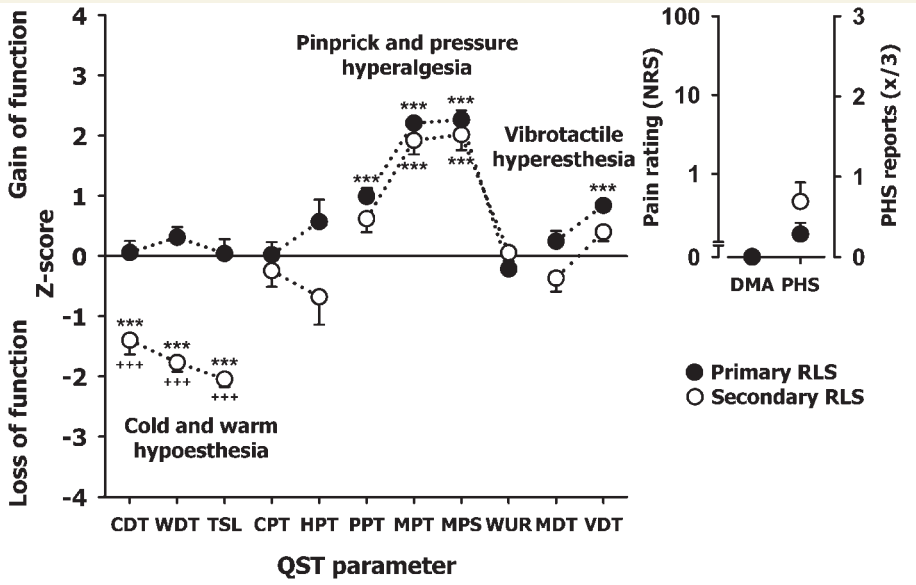


**Figure 1** Quantitative sensory testing data are presented as *z*-transformed sensory profiles. Compared with the quantitative sensory testing reference data of the German Research Network on Neuropathic Pain, negative *z*-scores indicate a loss of sensory function, while positive *z*-scores represent a gain of sensory function. Filled circles represent the data of $n = 21$ patients with primary RLS, open circles represent the data of $n = 13$ patients with secondary RLS associated with small fibre neuropathy. Both groups of patients with primary and secondary RLS showed a static mechanical hyperalgesia to pinprick stimuli. Idiopathic RLS patients exhibited blunt pressure hyperalgesia and vibrotactile hyperaesthesia. Patients with secondary RLS demonstrated increased thermal detection thresholds for the sensations of cold and warm, with the latter mediated by small fibre sensory afferents. Stars denote the level of significance as depicted from paired *t*-tests versus 0 ($^{***}P < 0.001$, Bonferroni-corrected for multiple comparisons). Crosses denote significant differences between idiopathic and secondary RLS patients ($^{+++}P < 0.001$), unpaired *t*-test, Bonferroni-corrected for multiple comparisons. All data are presented as mean ± SEM. QST = quantitative sensory testing; CDT = cold detection threshold; WDT = warm detection threshold; TSL = thermal sensory limen; CPT = cold-pain threshold; HPT = heat-pain threshold; PPT = pressure-pain threshold; MPT = mechanical pain threshold; MPS = mechanical pain sensitivity; WUR = wind-up ratio; MDT = mechanical detection threshold; VDT = vibration-detection threshold; DMA = dynamic mechanical allodynia; PHS = paradoxical heat sensation.

Ltd) at a magnification of 400. The epidermal length was measured using CellA® software (Soft imaging System, Münster, Germany). Intraepidermal PGP9.5-positive nerve fibres were counted according to the criteria described by Lauria *et al.* (2005). Values below 5 intraepidermal nerve fibres per millimetre (95th percentile) were considered indicative for small fibre neuropathy if electrophysiological measurements were largely normal (Kennedy *et al.*, 2004). Values between five and seven intraepidermal nerve fibres per millimetre were considered possible small fibre neuropathy. The neuropathologist assessing the skin biopsies (CS) was blinded to the clinical data.

# Results

## Clinical profile of patients with idiopathic versus secondary RLS associated with small fibre neuropathy

Based on their clinical symptoms, patients were diagnosed with primary RLS or secondary RLS associated with small fibre neuropathy. In the primary RLS group only one patient reported intermittent shooting pain, which was completely responsive to dopaminergic treatment with ropinirole. This patient also had a family history of RLS (Table 1). These findings suggest a diagnosis of primary RLS. Except for one patient who reported only numbness as a minus symptom, all patients in the secondary RLS group described persistent plus symptoms, mainly painful sensory symptoms, which were not, or only partly, responsive to dopaminergic medication. All patients in this group had preferentially responded to neuropathic pain medication (pregabalin, gabapentin, tilidine, etc.), some of them in combination with dopaminergic medication (Table 1). In two patients, effective treatment with pregabalin had to be discontinued due to pronounced side-effects (dizziness, increased daytime sleepiness, etc.), and one patient had not received any treatment (*de novo*). In all patients with secondary RLS associated with small fibre neuropathy, the diagnosis of small fibre neuropathy was reconfirmed by skin biopsy. The range of intraepidermal nerve-fibre densities in the distal leg segment among all patients examined ($n = 13$) was between 1.7 and 6.4 intraepidermal nerve fibres per millimetre. Patients with clinically diagnosed secondary RLS associated with small fibre neuropathy, had an average density of $3.6 \pm 1.7$ intraepidermal nerve fibres per millimetre. Patients with idiopathic RLS were significantly younger ($53.4 \pm 8.4$ years) than patients with secondary RLS ($63 \pm 8.2$ years; $P < 0.01$). The mean disease duration in patients with idiopathic RLS was $12.4 \pm 12$ years, whereas in patients with secondary RLS, the disease duration was $16.4 \pm 12.7$ years ($P = 0.4$). The mean score on the International Restless Legs Study Group Severity Scale was $29.7 \pm 6.3$ (mean $\pm$ SD) for patients with primary RLS and $26.9 \pm 5.9$ for patients with secondary RLS associated with small fibre neuropathy ($P = 0.2$). Laboratory testing showed normal serum levels of iron, ferritin, vitamin B12, vitamin B6, folic acid, creatinine and thyroid-stimulating hormone in patients with primary RLS. Similar results were obtained for patients with secondary RLS, although vitamin B6 serum concentrations were low (1.0 and 3.8 μg/l, normal range: 4–20 μg/l) in two secondary RLS patients (15%). Furthermore, among 13 patients with secondary RLS, seven patients (54%) and four patients with primary RLS (26%) showed an elevated glycosylated haemoglobin ($HbA_{1c}$; normal range: <6.1%), pointing to a diabetic aetiology of small fibre neuropathy in the secondary RLS group ($P < 0.05$ for difference between primary and secondary RLS). A positive family history was more prevalent in patients with idiopathic RLS (62%), whereas only 38% of patients with secondary RLS had a family history of RLS.

## Sensory profiling

The quantitative sensory testing protocol of the German Research Network on Neuropathic Pain consists of 13 parameters that allow assessment of the complete somatosensory phenotype. Figure 1 shows the sensory profiles of patients with idiopathic RLS and those of secondary RLS associated with small fibre neuropathy, each compared with published quantitative sensory testing reference data from 180 healthy control subjects from the German Research Network on Neuropathic Pain. In addition, a classical approach was performed using a second control group of 20 healthy control subjects that confirmed the main findings of hyperalgesia to pinprick in both patient groups, while patients with secondary RLS showed a profound loss of small fibre function (Table 2). Due to the overlapping evidence from two different control groups, the data comparing the quantitative sensory testing data of the German Research Network on Neuropathic Pain are shown in Fig. 1. Dynamic mechanical allodynia or increased temporal pain summation were not observed. Paradoxical heat sensations were only present in a minority of idiopathic RLS patients (in 4 of the 21 patients with primary RLS and in 7 of the 11 patients with secondary RLS).

## Idiopathic RLS

When compared with age- and gender-matched control subjects, patients with idiopathic RLS did not show minus signs (loss of sensory function) for any of the tested quantitative sensory testing parameters. Sensory plus signs were detected for mechanical pain and detection thresholds. Patients with idiopathic RLS demonstrated highly significant hyperalgesia for blunt pressure ($P < 0.001$) and pinprick stimuli ($P < 0.001$). Furthermore, increased pain ratings to suprathreshold mechanical stimuli were observed within an s/r-function for pinprick stimuli of different forces ($P < 0.001$). Patients with idiopathic RLS also showed a vibratory hyperaesthesia ($P < 0.001$). Interestingly, in idiopathic RLS, thermal detection thresholds were not altered, indicating the absence of small fibre dysfunction.

## Secondary RLS

Similar to patients with primary RLS, patients with secondary RLS associated with small fibre neuropathy demonstrated hyperalgesia to pinprick stimuli ($P < 0.001$). Furthermore, patients with secondary RLS also demonstrated significantly increased pain ratings to suprathreshold mechanical pain stimuli ($P < 0.001$), indicating a profound static mechanical hyperalgesia as also observed in patients with idiopathic RLS. In addition to evoked pain,

**Table 2 Quantitative sensory testing in RLS**

| Quantitative sensory testing (QST) | Idiopathic RLS (mean ± SD) | Secondary RLS (mean ± SD) | Controls (mean ± SD) |
|---|---|---|---|
| QST parameter (expressed in log) | | | |
| Cold detection threshold (°C log from BL) | 0.396 ± 0.239 | 0.807 ± 0.245 | 0.424 ± 0.294 |
| Warm detection threshold (°C log from BL) | 0.607 ± 0.173 | 1.059 ± 0.101 | 0.611 ± 0.206 |
| Thermal sensory limen (°C log) | 0.896 ± 0.235 | 1.355 ± 0.080 | 0.924 ± 0.203 |
| Cold-pain threshold (°C) | | | |
| Heat-pain threshold (°C) | | | |
| Pressure-pain threshold (kPa log) | 2.571 ± 0.087 | 2.597 ± 0.102 | 2.736 ± 0.209 |
| Mechanical pain threshold (mN log) | 0.993 ± 0.091 | 1.044 ± 0.200 | 1.737 ± 0.381 |
| Mechanical pain sensitivity (pain rating 0–100 log) | 0.949 ± 0.321 | 0.831 ± 0.435 | −0.075 ± 0.396 |
| Wind-up (ratio log) | 0.331 ± 0.162 | 0.373 ± 0.318 | 0.404 ± 0.314 |
| Mechanical detection threshold (mN log) | 0.269 ± 0.341 | 0.565 ± 0.398 | 0.374 ± 0.489 |
| Vibration-detection threshold (x/8) | | | |
| Dynamic mechanical allodynia (pain rating 0–100 log) | −1.000 ± 0.000 | −0.994 ± 0.022 | −1.000 ± 0.000 |
| Paradoxical heat sensations (x/3) | | | |
| QST parameter (expressed in original units)[a] | | | |
| Cold detection threshold (°C from BL) | −2.49 | −6.41 | −2.65 |
| Warm detection threshold (°C from BL) | 4.04 | 11.46 | 4.08 |
| Thermal sensory limen (°C) | 7.88 | 22.65 | 8.39 |
| Cold-pain threshold (°C) | 10.81 ± 8.70 | 7.67 ± 8.81 | 7.24 ± 8.70 |
| Heat-pain threshold (°C) | 44.36 ± 3.65 | 47.46 ± 2.74 | 46.53 ± 1.56 |
| Pressure-pain threshold (kPa) | 372 | 395 | 544 |
| Mechanical pain threshold (mN) | 10 | 11 | 55 |
| Mechanical pain sensitivity (pain rating 0-100) | 8.78 | 6.67 | 0.74 |
| Wind-up (ratio) | 2.14 | 2.36 | 2.54 |
| Mechanical detection threshold (mN) | 1.86 | 3.67 | 2.37 |
| Vibration-detection threshold (x/8) | 7.86 ± 0.34 | 7.13 ± 0.86 | 6.93 ± 1.23 |
| Dynamic mechanical allodynia (pain rating 0-100) | 0.000 | 0.001 | 0.000 |
| Paradoxical heat sensations (x/3) | 0.29 ± 0.64 | 0.69 ± 0.85 | 0.25 ± 0.64 |

Patient data were measured over the clinically more affected foot. BL = baseline 32°C; thermal detection thresholds are expressed as the difference (log and raw data) from this baseline temperature.
a Retransformed mean for log-normally distributed data.

11 patients with secondary RLS (85% of the secondary RLS patient group), but only one patient in the primary RLS group (5%), reported spontaneous pain ($P < 0.001$; Table 1). Thermal testing demonstrated a pronounced hypoaesthesia to cold (Aδ-fibre mediated) and warm (C-fibre mediated) stimuli (both $P < 0.001$).

# Discussion

The present study represents the first establishment of a comprehensive quantitative sensory test battery to identify distinct sensory profiles of primary and secondary RLS associated with small fibre neuropathy. Differentiation of restless legs subtypes is of utmost clinical relevance as it enables a differentiated therapeutic approach for patients with RLS. For the first time, we were able to show that patients with secondary RLS due to small fibre neuropathy showed significantly increased thermal detection thresholds as compared with patients with idiopathic RLS and healthy controls. Our study also shows for the first time that patients with primary RLS exhibit decreased vibration-detection thresholds and pressure-pain thresholds when compared with age- and gender-matched controls. Formerly published static mechanical hyperalgesia was confirmed for both RLS subtypes.

## Distinct sensory modality profile in patients with idiopathic RLS

Previously demonstrated static mechanical hyperalgesia was confirmed for patients with primary and secondary RLS (Stiasny-Kolster *et al.*, 2004). In accordance with Stiasny-Kolster *et al.* (2004), we observed a central sensitization or disinhibition as reflected by static mechanical hyperalgesia in both primary and secondary RLS patients. The majority of nociceptive Aδ- and C-fibres terminate in laminae I and II of the spinal dorsal horn, whereas Aß-fibres terminate in laminae III–IV (Todd, 2002). The activity of dorsal horn nociceptive transmission is regulated by descending tracts with inhibitory or excitatory influences (D'Mello and Dickenson, 2008). Following a peripheral nerve injury or inflammation, a shift in the balance of excitatory and inhibitory influence can develop (Wei *et al.*, 1999) and result in an increased response of spinal neurons to afferent input, a so-called central sensitization (Urban and Gebhart, 1999; D'Mello and Dickenson, 2008). A conversion of GABA-mediated

inhibition to excitation due to polarity inversion of $GABA_A$ receptor-mediated action on nociceptive spinal lamina I neurons may be associated with mechanical and thermal hyperalgesia in neuropathic animals (Sandkühler, 2009). Static mechanical hyperalgesia reflecting a central sensitization is a rather non-specific clinical symptom, which has also been reported in patients with migraine (LoPinto *et al.,* 2006), complex regional pain syndrome (Drummond *et al.,* 2006) and peripheral neuropathies (Baron, 2000; Moller *et al.,* 2006).

However, additional quantitative sensory testing parameters facilitated the establishment of a specific sensory profile for patients with RLS demonstrating further sensory abnormalities. We also observed decreased pressure-pain thresholds and a decreased vibration-detection threshold in patients with primary RLS. Vibration-detection threshold testing was performed with a Rydel–Seiffert graded tuning fork (64 Hz). A number of studies have shown that vibration of muscle tendons even at lower frequencies of 20–120 Hz (Fallon *et al.,* 2007) or 60 Hz (Verschueren *et al.,* 1998) is detected by muscle spindle receptors. Vibration results in a small variation of muscle length, thereby activating low-threshold muscle spindle proprioceptors. Decreased vibration-detection thresholds indicate an increased excitability of those non-pain-encoding muscle spindle proprioceptors, or of the respective neuronal relay stations of their central projection pathways. Similarly, significantly decreased pressure-pain thresholds suggest an increased excitability of high-threshold muscle pain afferents.

Our findings correspond with the pathophysiological concept of Clemens *et al.* (2006), who hypothesized that, in RLS, a dysfunction of the dopaminergic A11 neurons could shift the descending control to excitation with an increased sympathetic drive and increased norepinephrine. In line with this notion, several clinical studies reported an association of RLS and periodic limb movements in sleep with sympathetic overactivity (for a review, see Walters and Rye, 2009) and increased excitability of the spinal flexor reflex during sleep (Bara-Jimenez *et al.,* 2000).

## Differential diagnosis of primary versus secondary RLS

The present study suggests that thermal hypoaesthesia in patients with secondary RLS may differentiate primary RLS from secondary RLS due to small fibre neuropathy.

In line with our observations at clinical examination, the group of patients with secondary RLS associated with small fibre neuropathy showed a highly significant increase in thermal detection thresholds pointing to a significant deficit in small fibre function compared with controls and idiopathic RLS, as small fibres mediate thermal and nociceptive sensation (Devigili *et al.,* 2008). Schattschneider *et al.* (2004) assessed temperature perception with quantitative sensory testing in idiopathic and secondary RLS and observed an impaired temperature perception in 55% of idiopathic and 72% of secondary RLS patients. They evaluated only the function of C fibres with the quantitative nocioceptor axon reflex, which was impaired in patients with secondary RLS and intact in idiopathic RLS. However, the performance of small Aδ-fibres, which also mediate temperature sensation, was not objectively assessed. In the present study, we performed skin biopsies in patients with secondary RLS associated with small fibre neuropathy to quantify intraepidermal nerve-fibre density and confirmed the diagnosis of small fibre neuropathy with skin biopsies. A recent study by Devigili *et al.* (2008) demonstrated a significant diagnostic efficiency for skin biopsy (88.4%) in small fibre neuropathy. The increased thermal detection thresholds we observed may not be 100% specific and sensitive for small fibre neuropathy. Skin biopsies may not have been normal in all RLS patients if they have been performed in patients with primary RLS.

As no epidemiological studies with defined diagnostic criteria for small fibre neuropathy have been conducted so far, the prevalence of small fibre neuropathy in the general population has not been determined (for a review, see Lauria, 2005). This study identified 13 patients with small fibre neuropathy in 34 patients with RLS (38%). The percentage we observed is in-line with previously published studies, ranging from 45% of RLS patients with marked or borderline small fibre neuropathy (Polydefkis *et al.*, 1999) to 23% of RLS patients with isolated small fibre neuropathy or mixed with large fibre neuropathy (Polydefkis *et al.*, 2000).

Patients with secondary RLS due to small fibre neuropathy reported spontaneous pains or ongoing pain that was mostly described as a burning sensation. This finding is consistent with a neuropathic pain syndrome associated with restless legs in the group of the patients with secondary RLS. In contrast, only one patient with idiopathic RLS reported shooting pain, while paraesthesias were frequently present. This observation is in line with centrally and/or peripherally altered processing of sensory input and consistent with an absence of any relevant central or peripheral damage of the somatosensory system in idiopathic RLS.

Among the patients with secondary RLS associated with small fibre neuropathy, a positive family history was lower than in patients with idiopathic RLS (38% versus 62%) and similar to the previously published data of Hattan *et al.* (2009), who identified 37% patients with RLS and neuropathy reporting a positive family history. Previous investigations had shown slightly lower percentages of a positive family history in patients with secondary RLS and neuropathy, ranging from 27% (Merlino *et al.,* 2007), 25% (Iannaccone *et al.*, 1995) to 20% (Polydefkis *et al.*, 2000), which may be due to varying patient collectives with small and/or large fibre neuropathies of different aetiologies.

# Conclusions and clinical implications

In summary, this study shows that patients with secondary RLS with small fibre neuropathy have increased temperature-detection thresholds compared with idiopathic RLS and controls. Patients with idiopathic RLS showed hyperalgesia to blunt pressure and vibrotactile hyperaesthesia. Both RLS subtypes demonstrated a pinprick hyperalgesia. As patients were tested only in the presence of restless legs symptoms, we cannot exclude that the results may be different in symptom-free intervals. Our findings are compatible with either an impaired descending inhibition or an increased

spinal excitability in RLS patients. While no single test can detect the absence or presence of RLS, quantitative sensory testing is not only a supportive diagnostic tool that helps establish a specific sensory profile for the differential diagnosis of primary and secondary RLS, but also provides valuable data when choosing a specific treatment for subgroups of the disease.

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