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Biological mechanism of post-herpetic neuralgia: Evidence from multiple patho-psychophysiological measures

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Conflicts of interest

None declared.

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Abstract

Background: Post-herpetic neuralgia (PHN), which develops after the resolution of a herpes zoster eruption, is an exceptionally drug-resistant neuropathic pain. The unsatisfactory management of PHN partly results from the difficulty in dissecting out its contributing factors due to the complexity of PHN mechanism.

Methods: Here, to elaborate our understanding of the PHN mechanism and to establish a basis for effective therapeutic strategies, we comprehensively investigated the contributions of multiple factors to PHN severity.

Results: Based on the comparison of somatosensory detection thresholds (C, A δ and A β fibre thresholds) between affected and unaffected sides, 16 PHN patients with significant sensory deficits and 13 PHN patients without significant sensory deficits were identified and assigned to different groups. The different extents of lesions in the nociceptive system between patients with and without sensory deficits were confirmed using laser-evoked brain responses. Moreover, patients with sensory deficits had more severe pain and psychological disorders, e.g. anxiety and depression. Importantly, chronic pain severity was significantly influenced by various psychophysiological factors (sleep disturbances, psychological disorders and hypothalamic-pituitary-adrenal axis dysfunction) for patients with sensory deficits.

Conclusions: Our findings demonstrated the contribution of multiple patho-psychophysiological factors to PHN severity, which could help establish a basis for the development of a rational, patient-centred therapeutic strategy.

Significance: This study revealed the contribution of multiple patho-psychophysiological factors to PHN severity, which expanded our understanding of the underlying PHN mechanism, and helped develop a rational, patient-centred therapeutic strategy targeting towards the corresponding etiology and psychophysiological disorders for individual patient.

1. Introduction

As a prototypical human chronic neuropathic pain, post-herpetic neuralgia (PHN) is the most common sequela of acute herpes zoster infection. PHN patients are clinically characterized by spontaneous or evoked types of pain syndromes (e.g. sharp, stabbing and burning) (Pappagallo et al., 2000), and by the appearance of various abnormal sensory symptoms (e.g. hyperalgesia, allodynia and sensory loss) (Baron et al., 2010; Maier et al., 2010; Wang et al., 2011). In addition, PHN patients experience severe and prolonged psychophysiological problems, including sleep disturbances, anorexia and depression (Schmader, 2002; Katz et al., 2005; Oster et al., 2005; Johnson et al., 2010). Therefore, such chronic neuropathic pain has profound impact on patients' functional ability and quality of life, and importantly, remains resistant to current medical treatments, especially in developing countries.

Notwithstanding its well-recognized etiology, PHN manifests heterogeneous sensory signs and symptoms (Pappagallo et al., 2000; Maier et al., 2010). Specifically, several subtypes of PHN patients exist (Fields et al., 1998; Baron et al., 2010; Wang et al., 2011), including (1) patients with irritable nociceptors presenting stimulus-evoked symptoms of mechanical allodynia and, sometimes, thermal hyperalgesia; (2) patients with deafferentation presenting spontaneous pain and partial sensory deficits; and (3) patients with central reorganization presenting mechanical allodynia and sensory deficits. Even though multiple etiological mechanisms exist, the core pathological mechanism underlying PHN is a possible lesion in the afferent transmission system, which could result in minimal, partial, or complete loss of somatosensation. In addition, PHN is strongly associated with various psychophysiological disorders (Schmader, 2002; Katz et al., 2005; Oster et al., 2005; Johnson et al., 2010; Zeng et al., 2015). For example, PHN patients exhibit greater anxiety, depression, disease conviction, and lower life satisfaction during their acute herpes zoster stage than patients with herpes zoster who did not develop PHN (Katz et al., 2005; Volpi et al., 2008). This observation is strongly suggestive of a contribution of psychological factors to the development of PHN. In the meantime, due to the suffering from long-term psychological stress, the dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis is commonly observed in chronic pain patients (McBeth et al., 2005; Aloisi et al., 2011; Park and Ahn, 2012; Staufenbiel et al., 2013).

Because multiple contributing factors seem to determine the severity of PHN, the management of chronic pain requires an integrated approach, in which most of these contributing factors are considered. Based on the assessment of the contributing factors, pain relief could be optimally achieved by delivering multiple therapies, both pharmacological and non-pharmacological, in a rational, patient-centred manner. This strategy should not only evaluate and treat the primary pathological injury, but also identify and eliminate the possible psychophysiological factors responsible to the PHN syndrome.

To achieve a better understanding of PHN mechanisms, we comprehensively investigated the contribution of multiple factors to PHN severity using a descriptive approach. For each PHN patient, we evaluated possible lesions in somatosensory systems by measuring somatosensory detection thresholds (C, A δ and A β fibre thresholds) and laser-evoked brain responses, and assessed the relationships between the severity of chronic pain and various patho-psychophysiological factors, including sleep disturbances, psychological states and HPA axis function.

2. Materials and methods

2.1 Patients

Twenty-nine PHN patients (14 females: aged 62.2 ± 2.6 years; 15 males: aged 63.2 ± 2.6 years; mean $\pm$ SEM, same hereafter) were recruited from the Department of Pain Management, Daping Hospital (affiliated with the Third Military Medical University, Chongqing, China). The diagnosis was based on clinical symptoms (including medical history, typical scars, pain severity and types) (Fields et al., 1998) and was confirmed by two well-trained pain specialists. Exclusion criteria included histories of neurological diseases or dementias, psychiatric disorders, or inability to complete the testing procedures. All included patients reported pain that continued for at least 1 month after resolution of the herpes zoster eruption (Kost and Straus, 1996). All patients gave their informed consents, and the local ethics committee approved the study.

2.2 Measurement of chronic pain

To measure the severity and category of their predominant pain, all patients were instructed to complete the Short-Form McGill Pain Questionnaire (Dworkin et al., 2009). This questionnaire contains

three subscales, including (1) a pain rating index (PRI), which consists of 15 descriptors on a four-point Likert-type scale ranging from 0 (none) to 3 (severe); (2) a 10-cm visual analogue scale (VAS), which measures the intensity of averaged daily pain during the past 2 weeks; and (3) a present pain intensity (PPI) index ranging from 0 (no pain) to 5 (unbearable pain). For each patient, the location of shingles-affected skin areas were visually identified (affected side), and the mirror-image shingles-unaffected skin areas contralateral to the affected location were also identified as self-controls for the following analysis (unaffected side).

2.3 Assessment of lesions in the somatosensory system

To assess possible lesions in the somatosensory system, which are considered as the primary cause of PHN (Pappagallo et al., 2000), we compared detection thresholds of C, A δ , and A β fibres between the affected and unaffected sides. Additionally, the dysfunction in the nociceptive system was confirmed by assessing brain responses evoked by radiant-heat laser stimuli that were delivered, respectively, to the affected and unaffected skin areas.

2.3.1 Somatosensory detection thresholds

Radiant-heat laser stimuli were generated by an infrared neodymium yttrium aluminium perovskite (Nd:YAP) laser stimulator with a 1.34- μ m wavelength (Electronic Engineering). A He-Ne laser was directed to the area to be stimulated. The laser beam was transmitted via an optic fibre, and its diameter was set at 7 mm (38 mm²) by focusing lenses. The duration of each laser pulse was 4 ms. Using these parameters, laser pulses have been showed to effectively and directly activate small myelinated A δ fibres and unmyelinated C fibres in the most superficial skin layers in previous studies (Iannetti et al., 2008; Mouraux and Iannetti, 2009; Tu et al., 2016). After each stimulus, the target of the laser beam was shifted by at least 1 cm in a random direction to allow for passive skin cooling and to avoid nociceptor sensitization or injury. For the affected and unaffected sides, C and A δ fibre thresholds were individually estimated by delivering laser pulses to the respective skin areas (~16 cm²) using the following method: a stimulus energy of laser pulses, starting from 0.5 J and increased in 0.25-J increments until the detection of warmth and pain sensation was obtained. This procedure was repeated four

times, and the mean stimulus energies, corresponding to C and A δ fibre thresholds, were calculated.

Trans-cutaneous electrical stimuli were constant-current square-wave pulses with a duration of 0.5 ms delivered through a pair of electrodes placed on the skin areas of the affected or unaffected sides, separated by a 1-cm inter-electrode distance. Considering that small myelinated A δ fibres and unmyelinated C fibres have a higher electrical activation threshold than large-diameter A β fibres (Frahm et al., 2013), the electrical stimuli are expected to predominantly activate non-nociceptive A β fibres (Hu et al., 2011) and evoke tactile sensation. Similarly, A β fibre threshold was individually estimated by delivering electrical stimuli (starting from 0.5 mA and increasing in 0.1-mA increments until the detection of tactile sensation was obtained) to the skin areas of the affected and unaffected sides. This procedure was also repeated four times, and the mean stimulus intensities, corresponding to A β fibre threshold, were calculated.

For each patient and each sensory fibre, the influence of pathological injury (e.g. by shingles) was quantified using a percentage value, which was obtained by dividing the difference of detection thresholds between the affected and unaffected sides by the detection threshold of the unaffected side (i.e. sensory threshold abnormality). Patients with sensory deficits were expected to have an increased detection threshold on the affected side in comparison with the unaffected side, i.e. a positive percentage value. In contrast, patients without sensory deficits would have an identical or decreased detection threshold of the affected side compared to the unaffected side, i.e. a close-to-zero or negative percentage value. To discriminate patients with or without severe sensory deficits, we estimated the 95% confidence interval, within which the sensory deficits were not significant (the significance level was set at 0.05). This 95% confidence interval was obtained by calculating 1.96 standard deviations of all non-positive percentage values and their corresponding absolute values (to ensure that the mean of all adopted values was zero). To guarantee the sensitivity of identifying patients with sensory deficits, patients with a percentage value outside the 95% confidence interval for any sensory modality were assigned to Group A (with significant sensory deficits). Patients with percentage values inside the 95% confidence interval for all sensory modalities were assigned to Group B (without significant sensory deficits). This procedure is reasonable, since any measurement error of sensory thresholds would

decrease the sensitivity of detecting patients with sensory deficits. In other words, the possible measurement error would only increase the possibility of not detecting the real sensory deficit, but would not increase the possibility of detecting the false positive sensory deficit.

2.3.2 Laser-evoked brain responses

Before the electroencephalographic (EEG) data collection, the highest laser energy to be used in the laser evoked potential (LEP) experiment was individually determined by increasing the stimulus energy in 0.25-J increments, until a rating of 6 of 10 was obtained. For each patient, six levels of laser energy (in 0.25-J increments) were used, and 10 laser pulses of each energy level were delivered to each stimulation side (i.e. affected or unaffected side) for a total of 60 laser stimuli per side. The order of stimulus energies was pseudorandomized, and the inter-stimulus interval varied randomly between 10 and 15 s.

During the EEG data collection, patients were instructed to focus their attention on stimuli and to relax their muscles. EEG data were recorded using 64 Ag-AgCl scalp electrodes placed according to the International 10-20 system (Brain Products GmbH; pass band: 0.01–100 Hz; sampling rate: 1000 Hz). The nose was used as the reference, and impedances of all electrodes were maintained below 10 k Ω . Electro-oculographic signals were simultaneously recorded using surface electrodes to monitor ocular movements and eye blinks.

EEG data were processed using EEGLAB (Delorme and Makeig, 2004), an open source toolbox running in the MATLAB (MathWorks) environment. Continuous EEG data were bandpass filtered between 1 and 30 Hz. EEG epochs were extracted using a window analysis time of 3000 ms (from –1000 ms to 2000 ms), and were baseline corrected using the prestimulus interval. Trials contaminated by eye-blinks and movements were corrected using an independent component analysis algorithm (Delorme and Makeig, 2004). In all datasets, these independent components had a large electro-oculographic channel contribution and a frontal scalp distribution. To obtain a ‘homogeneous’ sample of patients, the EEG data were analysed by flipping the left and right electrodes of all patients whose shingle-affected skin areas were identified as being on the right side (Zhao et al., 2015).

For each patient and stimulation side, the average LEP waveform was obtained by averaging epochs across all stimulus energies. Single-patient average

waveforms were subsequently averaged to obtain group-level waveforms (for displaying purpose only), regardless of the height location of the PHN. Peak latencies and amplitudes of N2 and P2 waves, defined as the most negative and positive deflections between 150 and 700 ms after stimulus onset, respectively, were measured from the average LEP waveform (Cz-nose) of each patient.

Sensory stimuli (including nociceptive stimuli) could not only evoke phase-locked responses (e.g. event-related potentials, ERPs), but also induce non-phase-locked modulations of the magnitude of ongoing EEG oscillations, including event-related desynchronization (ERD) and synchronization (ERS) in different frequency bands (Pfurtscheller and Lopes da Silva, 1999; Mouraux et al., 2003; Hu et al., 2013). These non-phase-locked responses, which could capture important information particularly related to the cortical processing of nociceptive information (Mouraux et al., 2003; Raij et al., 2004; Ploner et al., 2006; Zhang et al., 2012; Hu et al., 2013), can be lost using the classical across-trial averaging in the time domain. To explore both phase-locked and non-phase-locked brain responses elicited by laser stimuli, a time-frequency analysis was adopted in our study. Specifically, a time-frequency distribution (TFD) of the EEG time course was obtained using a windowed Fourier transform with a fixed 250-ms Hanning window. For each time course, the windowed Fourier transform yielded a complex time-frequency estimate at each point of the time-frequency plane, extending from –1000 to 2000 ms (in 2-ms intervals) in the time domain, and from 1 to 30 Hz (in 1-Hz intervals) in the frequency domain. The resulting spectrogram represents the signal power as a joint function of time and frequency at each time-frequency point. The spectrogram was baseline-corrected (reference interval: –800 to –200 ms) at each frequency using the subtraction approach (Hu et al., 2014). In agreement with several previous studies (Mouraux et al., 2003; Iannetti et al., 2008), the laser stimuli elicited a large phase-locked response (‘LEP’: 100–800 ms, 1–10 Hz) at central-parietal electrodes (e.g. CPz) and a typical non-phase-locked response, i.e. a long-lasting decrease in power in the alpha band (‘ERD’: 600–1800 ms, 8–13 Hz), at contralateral central electrodes (e.g. C5 and C6 for right and left stimulation sides, respectively). Magnitudes of ‘LEP’ and ‘ERD’ were calculated by computing the mean of the 20% pixels displaying the highest increase (for ‘LEP’) or decrease (for ‘ERD’) in oscillatory power for each patient.

The features of laser-evoked brain responses, including N2 and P2 latencies and amplitudes, 'LEP' and 'ERD' magnitudes, were compared using a mixed-design analysis of variance (ANOVA), with two between-patient levels ('Group': A and B) and two within-patient levels ('Side': affected and unaffected). When the interaction was significant, post hoc Tukey's pairwise comparisons were performed.

2.4 Assessment of pain comorbidity and HPA axis function

Along with lesions in the somatosensory system, PHN is associated with dysfunctions in the limbic system and the HPA axis that may cause various mental health problems, including sleep disturbances (Volpi et al., 2008), anxiety and depression (Katz et al., 2005), and post-traumatic stress syndrome (McBeth et al., 2005; Aloisi et al., 2011). To assess the contribution of these factors to PHN, we (1) quantified the HPA axis function using blood tests of three typical adrenal-related hormones, i.e. aldosterone (Aldo), cortisol (CORT) and adrenocorticotropin (ACTH), in the morning hours (between 8:00 a.m. and 9:00 a.m.); (2) instructed the patients to rate the average daily sleep disturbance during the past 2 weeks on a five-point rating scale, ranging from 0 (best possible sleep) to 4 (worst possible sleep); (3) instructed the patients to complete the Self-rating Anxiety Scale (SAS) (Jegede, 1977) and Self-rating Depression Scale (SDS) (Gabrys and Peters, 1985) to assess their levels of anxiety and depression symptoms, respectively.

To assess the joint influence of patho-psychophysiological factors on chronic pain, multiple linear regression analysis based on a forward stepwise selection procedure was applied (Bendel and Afifi, 1977). In this analysis, chronic pain severity (indexed by PRI, VAS or PPI) was used as the dependent variable, and the lesion in somatosensory systems (C, A δ and A β fibre impairment), psychological disorders (SAS and SDS), sleep disturbances, HPA axis dysfunctions (Aldo, CORT, ACTH) were used as explanatory variables. The forward stepwise selection procedure started by including the explanatory variable that could mostly and significantly explain the dependent variable ($p < 0.05$), and adjusted by repeatedly adding other explanatory variable (if any) that could significantly improve the fitting of the model ($p < 0.05$), until none could improve the model. Prior to multiple linear regression analysis, all variables were checked on deviation from normality using Kolmogorov–Smirnov test

(Lillief, 1967), and no serious deviation from normality was observed for any variable ($p > 0.05$). In addition, no significant collinearity was detected among these explanatory variables (variance inflation factor was lower than 1.4 and tolerance statistics was larger than 0.7) (Field, 2000).

3. Results

3.1 Clinical diagnosis

All patients ($n = 29$) were diagnosed with definite unilateral PHN, involving the supraorbital territories (V1–V3) in two patients, cervical dermatomes (C2–C5) in three patients, thoracodorsal dermatomes (C6–C8) in three patients, upper thoracic dermatomes (T1–T8) in 13 patients, lower thoracic dermatomes (T9–T12) in seven patients, and lumbar dermatomes (L1–L5) in one patient. The severity of pain ranged from mild to severe, with average ratings of 6.0 ± 0.8 , 4.1 ± 0.4 and 2.0 ± 0.2 across all patients in the PRI, VAS and PPI subscales, respectively. In addition, the severity of pain for patients with different height locations of the PHN was summarized in Supporting Information Table S1. As revealed by one-way ANOVA, there was no significant difference of pain severity across these patients with PHN at different height locations (PRI: $F = 0.92$, $p = 0.48$, partial eta-squared $\eta_p^2 < 0.01$; VAS: $F = 0.64$, $p = 0.67$, $\eta_p^2 < 0.01$; PPI: $F = 0.65$, $p = 0.67$, $\eta_p^2 < 0.01$). It should be noted that none of the patients had stimulus-evoked pain symptoms (e.g. allodynia), since (1) patients of this type were not able to complete the whole experimental testing procedures due to some physical and/or psychological functioning limitations, and (2) patients with allodynia cannot bear the pain when assessing A β fibre threshold, which was achieved using transcutaneous electrical stimuli.

3.2 Lesions in the somatosensory system

3.2.1 Somatosensory detection thresholds

Based on the comparison of the somatosensory detection thresholds between the affected and unaffected sides, 16 patients with significant sensory deficits were identified and assigned to Group A, and 13 patients without significant sensory deficits were assigned to Group B (Fig. 1). Demographic (gender and age) and clinical (duration and severity of pain) characteristics of patients in Groups A and B were summarized in Table 1 and Supporting Information

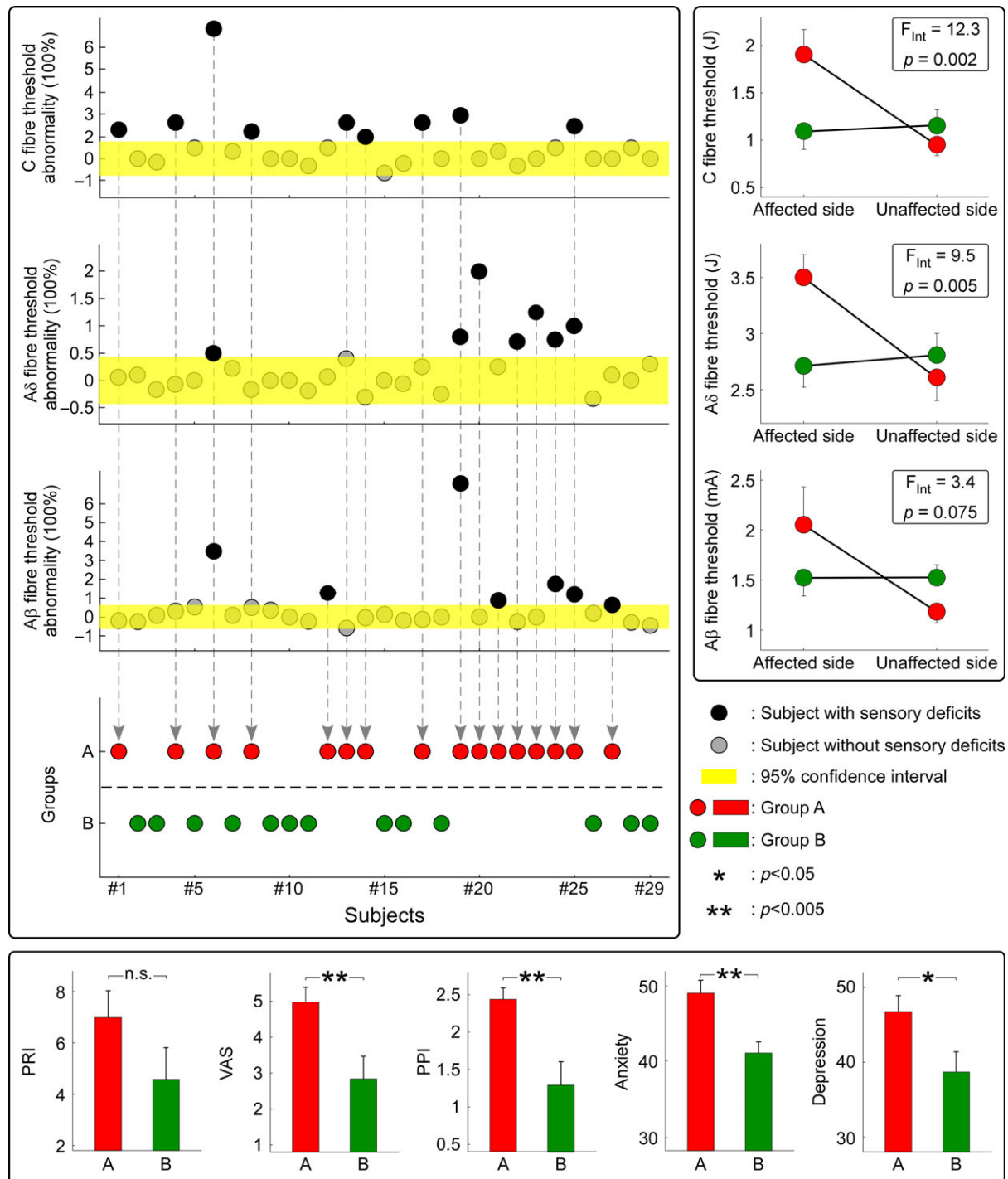


Figure 1 Somatosensory detection thresholds to determine the PHN patient subgroups. Top left: 16 patients with any significant sensory deficit (C, Aδ or Aβ fibre impairment) were assigned to Group A (indicated by red dots), and 13 patients without any significant sensory deficit were assigned to Group B (indicated by green dots). For each somatosensory detection threshold, the 95% confidence interval, within which the sensory deficit was not significant, was indicated by a yellow rectangle. Top right: Comparisons of somatosensory detection thresholds (C, Aδ or Aβ fibre thresholds) of patients in Groups A and B on the affected and unaffected sides. Significant interactions between factors 'Group' and 'Side' were observed for C and Aδ fibre thresholds but not for Aβ fibre threshold. Bottom: Comparisons of pain severity (i.e. PRI, VAS, and PPI) and psychological factors (i.e. SAS and SDS) between patients in Groups A and B. VAS, PPI, SAS, and SDS were significantly different between the two groups, while PRI was not.

Table 1 Demographic and clinical characteristics of patients in Groups A and B.

	Group A (n = 16)	Group B (n = 13)	p Value
Female/Male	7/9	7/6	–
Age (years)	63.1 ± 0.8	62.2 ± 3.1	0.81
Duration (months)	7.6 ± 0.9	8.1 ± 3.8	0.90
PRI	7.1 ± 1.1	4.6 ± 1.3	0.16
PRI-S	4.31 ± 0.59	3.30 ± 0.95	0.38
PRI-F	2.69 ± 0.56	1.08 ± 0.38	0.04
VAS	4.97 ± 0.42	2.84 ± 0.62	<0.01**
PPI	2.44 ± 0.15	1.29 ± 0.31	<0.01**
Sleep disturbance	2.31 ± 0.24	1.84 ± 0.28	0.23
SAS	49.1 ± 1.7	41.1 ± 1.5	<0.01**
SDS	46.8 ± 2.1	38.8 ± 2.7	0.03*
Aldo (ng/mL)	0.14 ± 0.45	0.15 ± 0.05	0.46
CORT (ng/mL)	174.3 ± 14.7	203.8 ± 26.9	0.33
ACTH (pg/mL)	6.58 ± 1.16	5.39 ± 0.71	0.43

PRI, Present pain intensity; PRI-S, Sensory subscale of PRI; PRI-F, Affective subscale of PRI; VAS, Visual analogue scale; PPI, Pain rating index; SAS, Self-rating Anxiety Scale; SDS, Self-rating Depression Scale; Aldo, Aldosterone; CORT, Cortisol; ACTH, Adrenocorticotropin.

* $p < 0.05$.

** $p < 0.01$.

Table S1. Whereas the patients' age and pain duration were not significantly different ($p > 0.05$ for both comparisons), the severity of pain, as quantified by VAS ($p = 0.01$) and PPI ($p < 0.01$), was significantly different between Groups A and B (independent sample *t*-test). However, the severity of pain, as quantified by PRI, was not significantly different between Groups A and B ($p = 0.16$, independent sample *t*-test). By separating the total PRI scores into sensory and affective subscales (Melzack, 1987; Ngamkham et al., 2012), i.e. PRI-S and PRI-F scores, we observed that between Groups, whereas PRI-S scores were not significantly different ($p = 0.38$, independent sample *t*-test), PRI-F scores were

significantly different ($p = 0.04$, independent sample *t*-test). These results indicated that the pain severity in the affective aspect was different between patients in Groups A and B, which could be served as a possible reason for significant differences of the present (PPI) and past (VAS) pain intensities between patients in Groups A and B.

The somatosensory detection thresholds (C, A δ and A β fibre thresholds) of patients in Groups A and B on the affected and unaffected sides (Table 2) were summarized and displayed in Fig. 1. As revealed by the mixed-design ANOVA, significant interactions between the factors 'Group' and 'Side' were observed for C fibre threshold ($F = 12.34$, $p < 0.01$, $\eta_p^2 = 0.25$) and A δ fibre threshold ($F = 9.52$, $p = 0.01$, $\eta_p^2 = 0.22$), but not for A β fibre threshold ($F = 3.42$, $p = 0.08$, $\eta_p^2 = 0.10$). *Post hoc* Tukey's pairwise comparisons revealed the within- and between-group differences, including (1) for patients in Group A, C and A δ fibre thresholds on the affected side were significantly greater than those on the unaffected side ($p < 0.01$ for both comparisons); however, for patients in Group B, neither threshold was significantly different between the affected and unaffected sides ($p = 0.67$ and $p = 0.46$, respectively); and (2) C and A δ fibre thresholds on the affected side were significantly greater for patients in Group A than those in Group B ($p = 0.03$ and $p = 0.01$, respectively), while C and A δ fibre thresholds on the unaffected side, as well as the A β fibre threshold on the affected and unaffected sides were not significantly different between two Groups ($p > 0.05$ for all comparisons).

In addition, we performed the same analysis by dividing PHN patients into two different groups: patients with small-fibre impairment were assigned to Group A' ($n = 13$); patients without small-fibre

Table 2 Somatosensory detection thresholds (C, A δ and A β fibre thresholds) on affected and unaffected sides and brain responses elicited by laser stimuli delivered to affected and unaffected sides for patients in Groups A and B.

	Group A		Group B	
	Affected side	Unaffected side	Affected side	Unaffected side
C fibre threshold (I)	1.91 ± 0.27	0.95 ± 0.12	1.10 ± 0.19	1.15 ± 0.17
A δ fibre threshold (I)	3.50 ± 0.21	2.61 ± 0.21	2.71 ± 0.19	2.81 ± 0.18
A β fibre threshold (mA)	2.05 ± 0.38	1.18 ± 0.11	1.52 ± 0.18	1.52 ± 0.13
N2 latency (ms)	434.08 ± 38.77	345.58 ± 40.02	360.88 ± 50.74	370.38 ± 57.23
N2 amplitude (μV)	−5.33 ± 0.98	−6.35 ± 1.15	−3.91 ± 1.24	−5.04 ± 0.89
P2 latency (ms)	659.42 ± 46.48	533.92 ± 46.89	644.13 ± 35.94	625.10 ± 37.29
P2 amplitude (μV)	5.26 ± 0.96	7.75 ± 1.38	7.13 ± 1.84	7.77 ± 1.71
'LEP' magnitude (μV)	0.43 ± 0.09	0.64 ± 0.14	0.38 ± 0.12	0.29 ± 0.08
'ERD' magnitude (μV)	−0.16 ± 0.05	−0.27 ± 0.06	−0.14 ± 0.06	−0.15 ± 0.06

LEP, Laser evoked potential; ERD, Event-related desynchronization.

impairment were assigned to Group B' ($n = 13$); patients with purely tactile abnormalities were dropped from the analysis ($n = 3$). Demographic (gender and age) and clinical (duration and severity of pain) characteristics of patients in Groups A' and B' were summarized in Supporting Information Table S2, and the somatosensory detection thresholds (C, A δ and A β fibre thresholds) of patients in Groups A' and B' on the affected and unaffected sides were summarized in Supporting Information Table S3. Note that the results obtained by the additional analysis were strikingly similar with the results that were obtained by dividing patients into Groups with or without any sensory deficits (Groups A and B), which

may indicate that PHN was similarly severe for patients with sensory deficits and patients with small-fibre impairment in the present study.

3.2.2 Laser-evoked brain responses

Group-level LEP waveforms (Cz-nose) and scalp topographies of N2 and P2 waves of patients in Groups A and B, evoked by laser stimuli delivered to affected and unaffected sides, were shown in Fig. 2 (left panel). LEP waveforms were clearly presented in 12 (of 16) patients in Group A and in 8 (of 13) patients in Group B. Peak latencies and amplitudes of N2 and P2 waves of both groups and sides

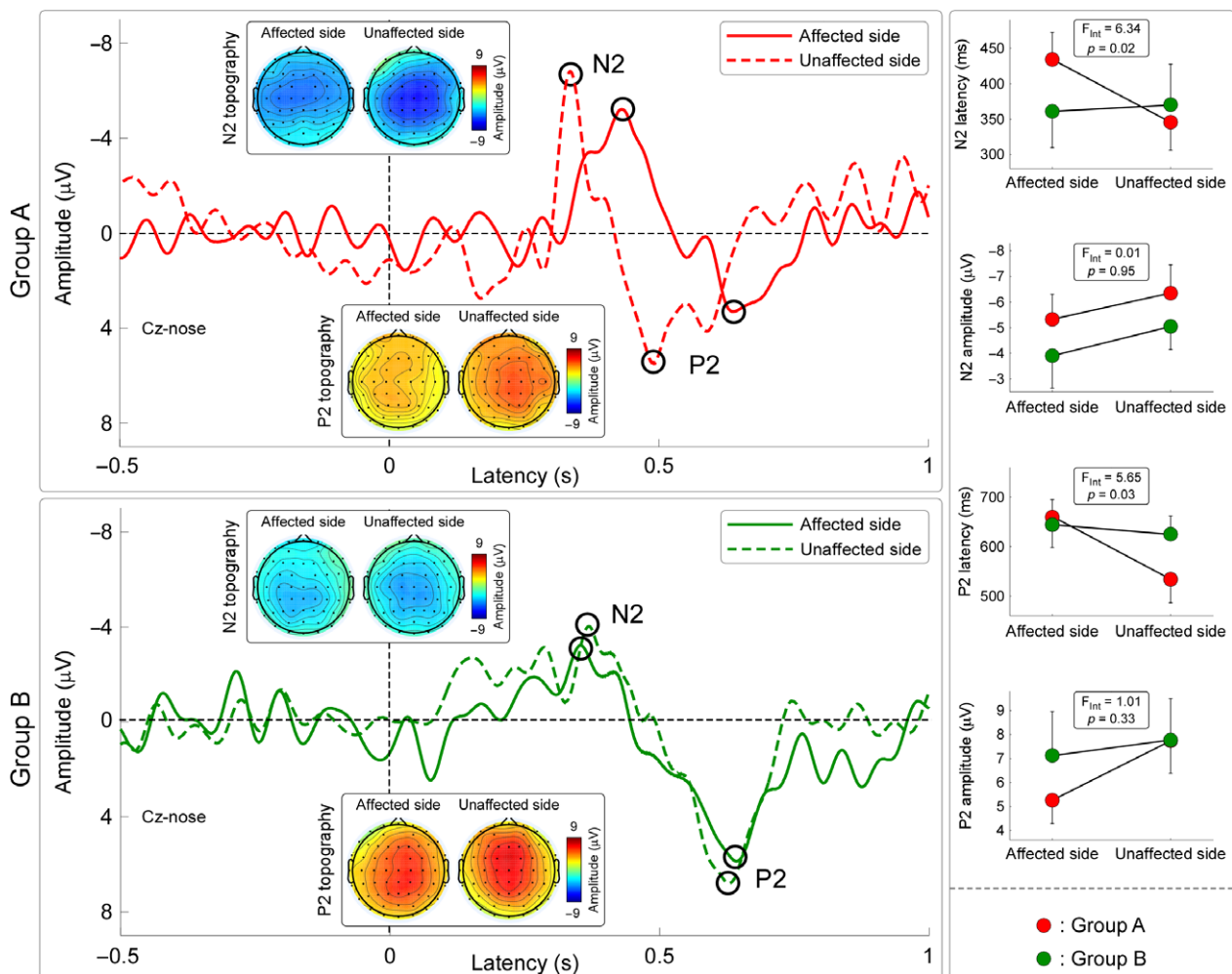


Figure 2 Group-level LEP waveforms of patients in Groups A and B. Left: Group-level LEP waveforms (Cz-nose) and scalp topographies of N2 and P2 waves of patients in Groups A (red) and B (green), evoked by the laser stimuli delivered to the affected (solid) and unaffected (dashed) sides, regardless of the height location of the PHN. For displaying purpose, group-level LEP waveforms were aligned to peak latencies of N2 and P2 waves. Right: Comparisons of latencies and amplitudes of N2 and P2 waves elicited by laser stimuli delivered to affected and unaffected sides for patients in Groups A and B. Significant interactions between factors 'Group' and 'Side' were observed for N2 and P2 latencies but not for N2 and P2 amplitudes.

(Table 2) were summarized and displayed in the right panel of Fig. 2. As revealed by the mixed-design ANOVA, significant interactions between the factors 'Group' and 'Side' were observed for N2 and P2 latencies ($F = 6.34$, $p = 0.02$, $\eta_p^2 = 0.20$ and $F = 5.56$, $p = 0.03$, $\eta_p^2 = 0.17$, respectively), but not for N2 and P2 amplitudes ($F = 0.01$, $p = 0.95$, $\eta_p^2 < 0.01$ and $F = 1.01$, $p = 0.33$, $\eta_p^2 = 0.05$, respectively). *Post hoc* Tukey's pairwise comparisons revealed both within- and between-group differences, including (1) for patients in Group A, N2 and P2 latencies on the affected side were significantly longer than those on the unaffected side ($p = 0.02$ and $p < 0.01$, respectively); for patients in Group B, N2 and P2 latencies were not significantly different between affected and unaffected sides ($p = 0.38$ and $p = 0.42$, respectively); and (2) N2 and P2 latencies and amplitudes were not significantly different between two Groups, no matter the stimuli were delivered on the affected or unaffected side ($p > 0.05$ for all comparisons). Even not statistically significant, N2 and P2 latencies at the unaffected side were relatively shorter for patients in Group A than in Group B ($p = 0.73$ and $p = 0.20$, respectively). This observation could be explained by the fact that the stimulation territory was relatively higher for patients in Group A than in Group B (Supporting Information Table S1).

In addition, abnormalities of N2 and P2 latencies (evaluated by differences in N2 and P2 latencies between affected and unaffected sides) were significantly greater for patients in Group A than those in Group B (N2 latency: 88.50 ± 29.44 ms vs. -9.50 ± 9.65 ms, $p = 0.02$; P2 latency: 125.50 ± 31.78 ms vs. 19.13 ± 20.78 ms, $p = 0.03$). In contrast, abnormalities of N2 and P2 amplitudes (evaluated by differences in N2 and P2 amplitudes between affected and unaffected sides) were not significantly different between two Groups (N2 amplitude: 1.02 ± 1.14 μ V vs. 1.14 ± 1.13 μ V, $p = 0.95$; P2 amplitude: -2.48 ± 1.36 μ V vs. -0.64 ± 0.61 μ V, $p = 0.33$).

Group-level TFDs (Cz-nose) of patients in Groups A and B, elicited by laser stimuli delivered to the affected and unaffected sides, were shown in Fig. 3 (top panel). For each group and side, laser stimuli elicited a large phase-locked response ('LEP', maximal at CPz-nose) and a typical non-phase-locked response ('ERD', maximal at C5 and C6 for right and left stimulation sides, respectively), and the magnitudes of both responses (Table 2) were summarized and displayed in the bottom panel of Fig. 3. As revealed by the mixed-design ANOVA, significant interactions between the factors 'Group' and 'Side'

were observed for the 'LEP' and 'ERD' magnitudes ($F = 5.73$, $p = 0.02$, $\eta_p^2 = 0.19$ and $F = 5.16$, $p = 0.03$, $\eta_p^2 = 0.19$, respectively). *Post hoc* Tukey's pairwise comparisons revealed that, for patients in Group A, 'LEP' and 'ERD' magnitudes on the affected side were significantly smaller than those on the unaffected side ($p = 0.04$ and $p = 0.01$, respectively); for patients in Group B, 'LEP' and 'ERD' magnitudes were not significantly different between the affected and unaffected sides ($p = 0.17$ and $p = 0.63$, respectively). Between-group comparisons showed that the 'LEP' magnitude on the unaffected side was significantly greater for patients in Group A than those in Group B ($p = 0.04$), while 'LEP' magnitude on the affected side, 'ERD' magnitudes on both affected and unaffected sides showed no significant difference between two Groups ($p > 0.05$ for all comparison). The weak difference between Groups could be explained by the fact that the delivered stimulus intensity was individually adjusted for each patient to ensure that the perceived pain at the unaffected side was similar across different patients (see Section 2.3.2).

In addition, the abnormality of 'LEP' magnitude (evaluated by the difference of 'LEP' magnitude between affected and unaffected sides) was significantly greater for patients in Group A than those in Group B (0.28 ± 0.12 μ V vs. -0.10 ± 0.06 μ V, $p = 0.02$, independent sample *t*-test), and the abnormality of 'ERD' magnitude (evaluated by the difference of 'ERD' magnitude between affected and unaffected sides) was marginally significantly different between two Groups (-0.10 ± 0.04 μ V vs. -0.01 ± 0.02 μ V, $p = 0.05$, independent sample *t*-test).

Correlation analysis across all patients showed that the 'ERD' magnitude on the unaffected side was significantly and negatively correlated with anxiety ($r = -0.46$, $p = 0.02$) and depression scores ($r = -0.66$, $p < 0.001$), and the 'ERD' magnitude on the affected side was significantly and negatively correlated with depression score ($r = -0.53$, $p < 0.01$). In addition, the abnormality of 'ERD' magnitude was significantly and negatively correlated with PRI ($r = -0.41$, $p = 0.03$) and anxiety score ($r = -0.52$, $p = 0.01$).

3.3 Relationship between chronic pain and psychophysiological factors

Psychophysiological factors of chronic pain, including sleep disturbances, psychological disorders (assessed by SAS and SDS scores), and adrenal-related

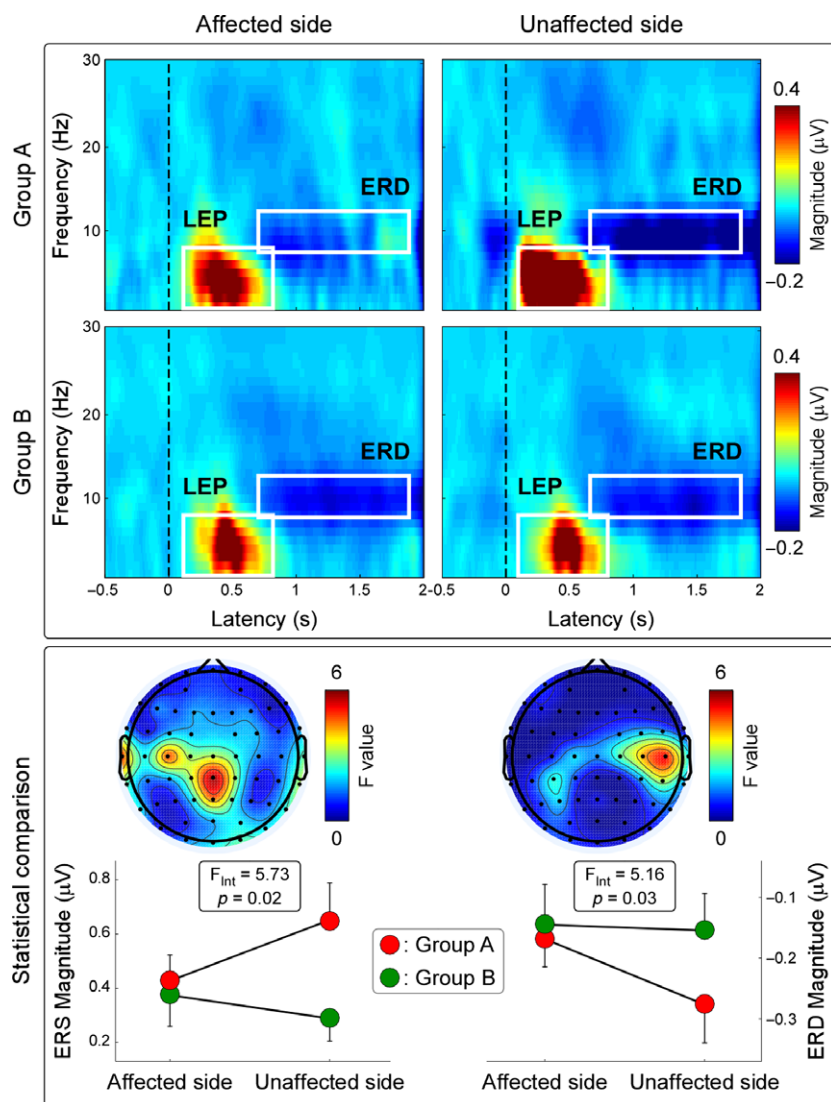


Figure 3 Group-level laser-elicited time-frequency distributions of patients in Groups A and B. Top: Group-level TFDs (Cz-nose) of patients in Groups A and B elicited by laser stimuli delivered to affected and unaffected sides. Laser-elicited TFDs contained a phase-locked response ('LEP': 100–800 ms, 1–10 Hz) and a non-phase-locked response ('ERD': 600–1800 ms, 8–13 Hz). Both regions of interest were indicated by white rectangles. Bottom: Comparisons of 'LEP' and 'ERD' magnitudes elicited by laser stimuli delivered to affected and unaffected sides for patients in Groups A and B. Significant interactions between factors 'Group' and 'Side' were observed for both 'LEP' and 'ERD' magnitudes, which were maximal at CPz and C5/C6, respectively.

hormones (Aldo, CORT and ACTH) for patients in Groups A and B, were summarized in Table 1. As revealed by the independent sample *t*-test, whereas sleep disturbance and adrenal-related hormones were not significantly different between patients in Groups A and B ($p > 0.05$ for all comparisons), psychological disorders, as measured by SAS ($p < 0.01$) and SDS ($p = 0.03$) scores, were significantly different (Fig. 1).

For Group A, multiple linear regression analysis revealed that (1) the dependent variable PRI was

significantly influenced by the combination of two explanatory variables (accounting for 66.3% of the variability; $F = 15.76$, $p < 0.001$): ACTH (standardized $\beta = 0.34$, $t = 2.56$, $p = 0.03$) and sleep disturbances (standardized $\beta = 0.56$, $t = 3.74$, $p < 0.01$); (2) the dependent variable VAS was significantly influenced by the combination of two explanatory variables (accounting for 61.4% of the variability; $F = 12.93$, $p < 0.001$): Aldo (standardized $\beta = -0.67$, $t = -3.69$, $p < 0.01$) and SAS (standardized $\beta = 0.88$, $t = 4.83$, $p < 0.001$); (3) the dependent variable PPI

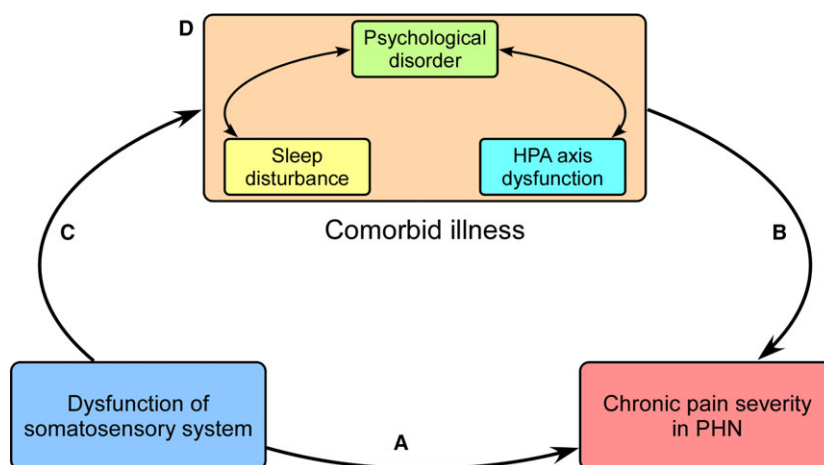


Figure 4 A schematic to illustrate the contributions of multiple factors to PHN. Arising from a possible sensory deficit of the afferent transmission system, PHN patients were normally characterized by heterogeneous sensory abnormalities and different types/levels of unrelenting pain (A). As revealed by multiple linear regression analysis, chronic pain severity was significantly influenced by the combination of multiple psychophysiological factors (i.e. sleep disturbances, psychological disorders and HPA axis dysfunctions) (B). Moreover, the possible influence of lesions in the somatosensory system on chronic pain patients' psychophysiological states (i.e. anxiety and depression) was inferred from the close relationships between sensory threshold abnormalities (as well as abnormalities of laser-induced 'ERD' magnitude) and psychological disorders (C). Additionally, interactions between these psychophysiological factors (D), e.g. blood level of CORT and SDS score, sleep disturbance and SAS score, were observed based on their significant correlations.

was significantly influenced by the explanatory variable (accounting for 27.6% of the variability; $F = 6.72$, $p = 0.02$): SAS (standardized $\beta = 0.57$, $t = 2.63$, $p = 0.02$) (Fig. 4). These results indicated that the chronic pain severity was associated with increased anxiety score, blood level of ACTH, and sleep disturbance, as well as decreased blood level of Aldo. In contrast, the chronic pain severity was not significantly influenced by the explanatory variables or their combinations for patients in Group B.

4. Discussion

By comparing somatosensory detection thresholds (C, A δ and A β fibre thresholds) between affected and unaffected sides, 16 PHN patients with significant sensory deficits and 13 PHN patients without significant sensory deficits were identified and assigned to Groups A and B, respectively (Fig. 1). Different levels of lesions in the nociceptive somatosensory system between patients in Groups A and B were confirmed by laser-evoked brain responses (Figs. 2 and 3). Patients in Group A showed more severe pain and psychological disorders (i.e. greater anxiety and depression) than patients in Group B (Fig. 1). By assessing the relationships between chronic pain severity and various psychophysiological factors, patients in Group A showed that chronic pain severity could be significantly

influenced by the combination of sleep disturbance, psychological disorder (i.e. anxiety scores) and HPA axis dysfunction (i.e. ACTH blood level) (Fig. 4). These results demonstrated the contribution of multiple factors to PHN severity, resulting in the notorious difficulty in managing this type of neuropathic pain. In addition to expanding the current understanding of the underlying PHN mechanism, our findings may aid in the recognition of multiple causes and contributing factors of PHN in clinical practice. This is important, as it would help develop a rational, patient-centred therapeutic strategy that is targeted towards the corresponding etiology and psychophysiological disorders for individual patient, based on the systematic clinical examination.

4.1 PHN etiology

Several studies have demonstrated that PHN patients manifest heterogeneous patterns of sensory abnormalities (Pappagallo et al., 2000; Maier et al., 2010). Specifically, different levels of somatosensory deficits caused by the reactivation of latent varicella zoster virus were considered to play a primary role in the development of PHN (Kost and Straus, 1996; Baron et al., 2010), and suggested to be associated with different pathological mechanisms of this chronic pain (Jensen and Baron, 2003). To explore the contribution of somatosensory deficits to PHN severity,

we assessed lesions in the somatosensory system by comparing the somatosensory detection thresholds (C, A δ and A β fibre thresholds) between affected and unaffected sides. We observed that approximately 55% of our cohort of PHN patients (16 of 29) had severe sensory deficits (i.e. significant increase in the detection threshold in at least one sensory modality on the affected side compared to the unaffected side), and approximately 45% of the patients (13 of 29) had either no or minimal sensory deficits (Fig. 1). Thereby, we divided all PHN patients into two groups (Group A: with significant sensory deficits; Group B: without significant sensory deficits). By comparing somatosensory detection thresholds (C, A δ and A β fibre thresholds) of patients in Groups A and B on the affected and unaffected sides, we observed that C and A δ fibre thresholds on the affected side were significantly greater than those on the unaffected side for patients in Group A but not for patients in Group B (Fig. 1). This observation indicated the abnormalities of C and A δ fibre thresholds for patients in Group A, thus demonstrating the impairment of the ascending nociceptive pathways that consist of slightly myelinated A δ and unmyelinated C spinothalamic fibres (Iannetti et al., 2008).

The lesion in the nociceptive system of patients in Group A was further confirmed by laser-evoked brain responses, which showed delayed N2 and P2 latencies (Fig. 2), as well as reduced phase-locked 'LEP' and non-phase-locked 'ERD' magnitudes when laser stimuli were delivered to the skin on the affected side, compared to the unaffected side (Fig. 3). In contrast, patients in Group B did not show any abnormality in these brain responses (Figs. 2 and 3). The delayed LEP latencies and attenuated 'LEP'/'ERD' magnitudes for patients in Group A would indicate the lesion in the nociceptive system that is mediated by small myelinated A δ fibres, possibly originating from (1) the demyelination of the fibres (Kimura, 1975), (2) the degeneration of dorsal root ganglion cells (Truini et al., 2008), or (3) the inhibition/impairment of central pain transmission neurons (Cruccu et al., 2001). However, it looks at odds that significant differences between affected and unaffected sides were not found for N2 and P2 amplitudes ($p = 0.41$ and $p = 0.11$, respectively) for patients in Group A (Fig. 2). The controversy between the results of N2/P2 amplitudes and 'LEP' magnitudes (measured the same information as N2-P2 waveforms did, but in the time-frequency domain) could be explained by the following two reasons. First, the measurement of N2 and P2 amplitudes would be easily influenced by some noise in

the time domain (e.g. time domain drift of the signal), and such influence could be greatly reduced if the signal was analysed using time-frequency analysis and time-frequency baseline-correction (considering that the possible signal drift would not be time-locked to the stimulus onset, it would be presented not only in the post-stimulus interval, but also in the pre-stimulus interval. For this reason, baseline-correction in the time-frequency domain would reduce the magnitude related to the signal drift). Second, the measurement of N2 and P2 amplitudes would also be easily influenced by the within-subject latency jitter of single-trial LEPs, while the measurement of 'LEP' magnitude in the time-frequency domain would not be influenced by such latency jitter considering that time-frequency representations would capture both phase-locked and non-phase-locked brain responses. In our study, the latency jitter of single-trial LEPs would be large, since six levels of laser energy (in 0.25-J increments, ten trials for each energy level) were used for each patient. Such latency jitter would lead to a distortion of the averaged LEPs (Mouraux and Iannetti, 2008), resulting in an underestimation of the amplitudes of LEP waveforms and even making LEPs undetectable in the time domain. An alternative way to improve the detection of laser-evoked brain responses would be the use of the global field power, which is a valuable summary of multi-channel EEG data (Lehmann and Skrandies, 1980). However, the results of global field power were not reported in the present study since global field power is a biased statistic (Files et al., 2016), because additional noise has a tendency to increase its value. Instead, the measurement of N2 and P2 latency and amplitude is highly recommended for various clinical applications (Treede et al., 2003), thus enabling us to compare results among different studies.

Importantly, 'LEP' and 'ERD' magnitudes on the unaffected side were likely greater for patients in Group A than Group B (Fig. 3). These observations would be associated with significant differences in pain severity (as quantified by VAS and PPI) and psychological disorders (i.e. anxiety and depression) between the two Groups (Fig. 1), as they may imply that the psychophysiological state in the central neural system was not the same between Groups. In other words, the obtained neurophysiological differences were not only determined by peripheral factors, e.g. lesions in the peripheral sensory system, but also influenced by central factors, e.g. brain states.

In the present study, LEP responses were not clearly presented in some patients (four patients in Group A and five patients in Group B), and N2 and P2 latencies after stimulation of the unaffected side were longer than their normal values. These phenomena could be explained by the following three reasons: (1) lesions in the nociceptive system of PHN patients (Nurmikko and Bowsher, 1990; Oaklander et al., 1998; Cruccu et al., 2001, 2003; Truini et al., 2003, 2008); (2) degenerative changes of the nociceptive pathway mainly consisting of axonal loss, as well as slowed cognitive processing of noxious information, with advancing age (Gibson et al., 1994; Gagliese and Melzack, 2000; Gibson and Helme, 2001; Truini et al., 2005); (3) the relatively low intensity of some of the delivered laser stimuli (Cruccu et al., 2003; Iannetti et al., 2004), which would not be high enough to activate A δ nociceptors in the superficial skin layers for some patients.

4.2 Psychophysiological contributors of PHN

In addition to the primary etiological cause, PHN, as a typical chronic neuropathic pain, has profound and prolonged psychophysiological consequences (Mcgrath and Dade, 2004; van Seventer et al., 2006), such as increased anxiety and depression (Volpi et al., 2008), as well as decreased health-related quality of life (Oster et al., 2005). Consistent with these concepts, we found that (1) psychological disorders, as measured by SAS and SDS scores, were significantly different between patients in Groups A and B (Fig. 1, $p < 0.01$ and $p = 0.03$, respectively, independent sample t -test); and (2) chronic pain severity for patients in Group A was associated with increased anxiety score, blood level of ACTH, and sleep disturbance, as well as decreased blood level of Aldo, as revealed by multiple linear regression. Notably, many types of chronic pain have been well documented to be highly associated with sleep disturbances and psychological disorders (Fishbain et al., 1997; Gore et al., 2005; Tsang et al., 2008; Schlereth et al., 2015). Moreover, the relationship between chronic pain and the blood level of adrenal-related hormones could reflect the effects of long-term stress related to chronic pain on HPA axis function (Aloisi et al., 2011). These observations highlighted the complexity of PHN, which would result in difficulties in the diagnosis and management of this chronic pain (Dworkin et al., 2003; Baron et al., 2010; Morales-Espinoza et al., 2015).

Although the causal relationship between chronic pain and psychophysiological disorders could not be

quantitatively identified in the present study, we speculated that at the acute herpes zoster stage, severe pain resulting from a herpes zoster eruption could lead to a radical disturbance of daily life (e.g. sleep disturbance) and various psychophysiological problems (Volpi et al., 2007; Drolet et al., 2010), such as insomnia, anxiety, or depression, which could be related to HPA axis dysfunction (unpublished data from JC's laboratory). These psychophysiological disorders, which were considered to be comorbidities of chronic pain, could aggravate the severity of pain during its chronicity process (Oster et al., 2005; van Seventer et al., 2006; Volpi et al., 2008). Indeed, the reciprocal relationship between chronic pain and its comorbidities could be further investigated using longitudinal studies (Fishbain et al., 1997; Mossey and Gallagher, 2004; Tunks et al., 2008; Kroenke et al., 2011). It should be noted that in clinical situations, difficulties of relieving pain without eliminating its comorbidities have been increasingly recognized (Fishbain, 1999; Mcgrath and Dade, 2004; Ballantyne and Sullivan, 2015; Morales-Espinoza et al., 2015; Dale and Stacey, 2016).

4.3 Limitations

Our study has two limitations. First, the sample size of patients is limited, which hampered the investigation of the detailed role of each mechanism in the development of PHN. Indeed, the causal relationship between patho-psychophysiological factors and chronic pain severity should be investigated not only based on a large sample of patients, but also using some effective experimental designs, e.g. a longitudinal study to monitor the transition from acute to chronic pain (Fishbain et al., 1997; Mossey and Gallagher, 2004; Tunks et al., 2008; Kroenke et al., 2011). Second, all patients included in the present study reported pain that continued for at least one month after healing from the shingle eruptions. Even though in some other studies, PHN was diagnosed when pain persisted one month after rash onset or healing (Rowbotham and Fields, 1996; Opstelten et al., 2002; Parruti et al., 2010; Whitley et al., 2010; Guan et al., 2015), many studies defined PHN as pain persisting beyond 3 or 6 months after rash onset or healing (Oaklander et al., 1998; Pappagallo et al., 2000; Bowsher, 2003; Truini et al., 2008; Maier et al., 2010). Whether the obtained findings in the present study could also be applied to PHN patients reporting pain beyond 3 or 6 months after rash onset or healing should be verified in the future.

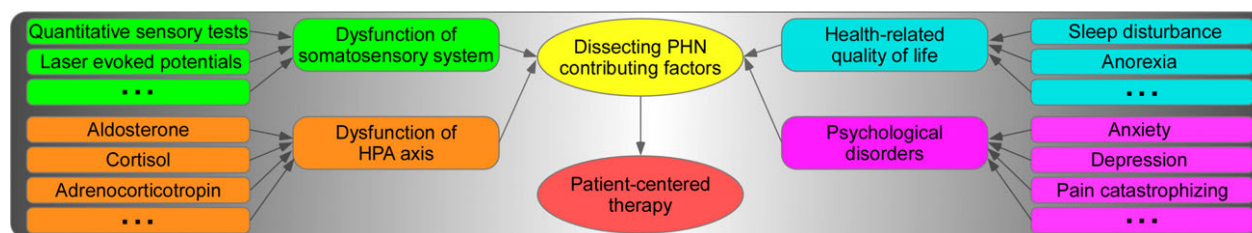


Figure 5 Flow chart describing the systematical diagnosis of PHN for patient-centred therapy. The systematical diagnosis of PHN includes, but not limits to, the examination of (1) possible lesions in the somatosensory system using quantitative sensory tests (e.g. C, A δ and A β fibres) and laser evoked brain responses; (2) health-related quality of life, such as sleep disturbance and anorexia; (3) possible dysfunction of HPA axis by evaluating the blood levels of adrenal-related hormones; and (4) psychological disorders, such as anxiety, depression and pain catastrophizing. With the dissection of contributing factors for PHN, patient-centred therapy, using multidisciplinary therapeutic approaches (including pharmacological and non-pharmacological treatment regimens), could be individually designed for optimizing the treatment effect.

4.4 Clinical implications

We provided direct evidence showing the multifactorial determinants of PHN, indicating that PHN should be considered as a clinical entity rather than a single disease state (Fig. 4). This would thereby expand our understanding of the biological mechanisms of PHN, and also contribute towards establishing a basis for patient-centred therapeutic strategy, i.e. prevention and treatment based on a systematic diagnosis for each patient. The systematic diagnosis includes, but not limits to, testing possible sensory abnormalities, health-related quality of life (e.g. sleep quality), psychological disorders and HPA axis dysfunction. The systematic diagnosis is important, as it allows for the comprehensive evaluation of the pathological and psychophysiological states for each chronic pain patient (Mcgrath and Dade, 2004; Baron et al., 2010). Following the thorough diagnosis, optimal treatment strategies for each PHN patient should be individually designed, not only based on the possible lesions in somatosensory systems, but also considering possible comorbidities associated with chronic pain (Fishbain, 1999; Nicholson and Verma, 2004; Morales-Espinoza et al., 2015). Ideally, a multidisciplinary therapeutic approach (Fig. 5), including pharmacological and non-pharmacological treatment regimens (e.g. cognitive behavioural, physical, and occupational therapies) should be individually designed to relieve pain for each patient, based on the dissection of the biological mechanism underlying PHN.

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Author contributions

WWP, XLG, JSW, JC and LH conceived and designed the experiment; QQJ, HW, XLX, YZ, PCH, WCW and SLL performed the experiment; WWP, XLG, QQJ, HW, XLX and LH analysed the data; WWP, XLG, JSW, JC and LH wrote the article.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. The number of patients and their pain severity for the different height location of the PHN.

Table S2. Demographic and clinical characteristics of patients in Groups A' and B'.

Table S3. Somatosensory detection thresholds (C, A δ and A β fibre thresholds) on affected and unaffected sides and brain responses elicited by laser stimuli delivered to affected and unaffected sides for patients in Groups A' and B'.