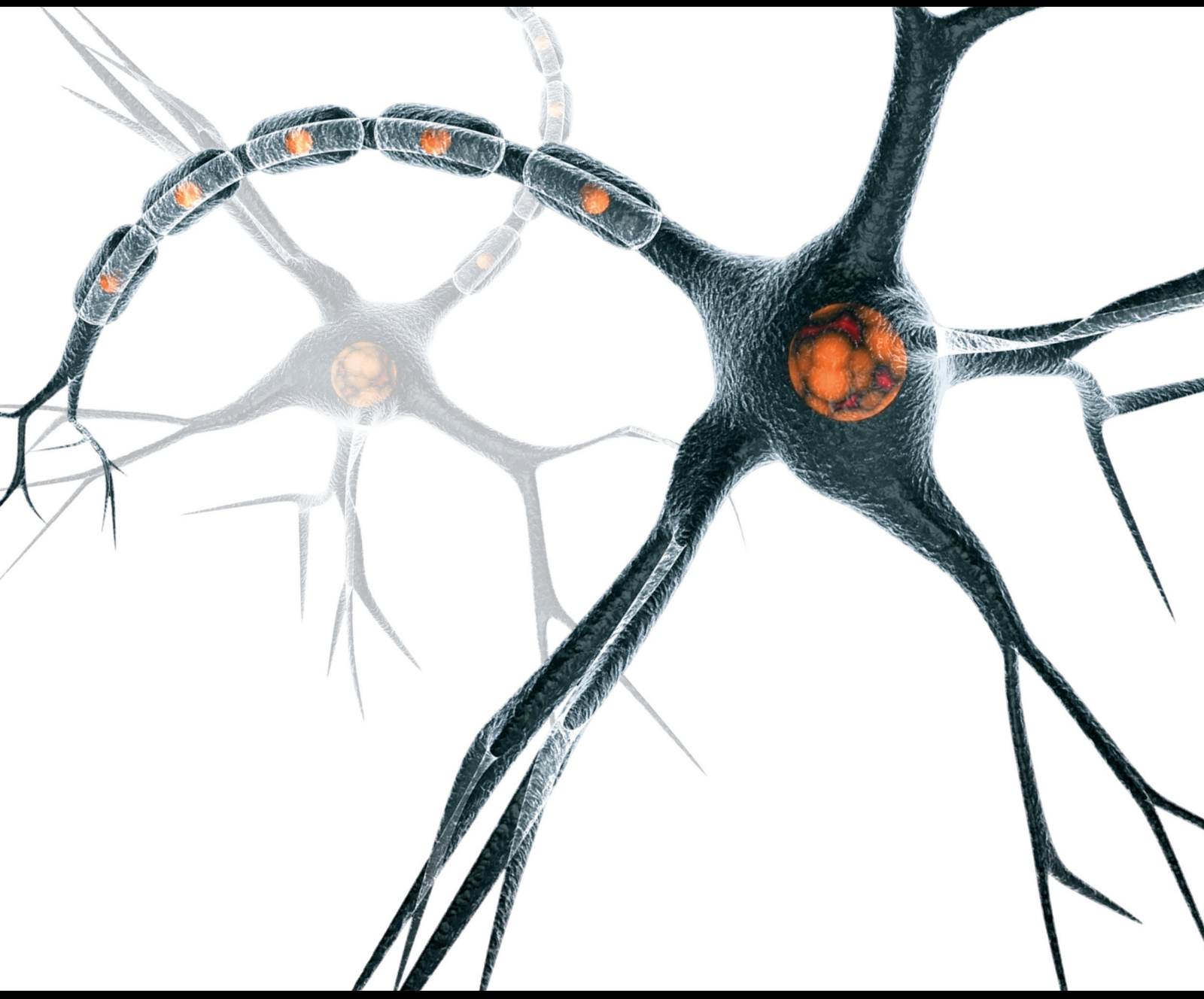
Neural Plasticity and Neuropathic Pain

Lead Guest Editor: Xue-Qiang Wang
Guest Editors: Yazhuo Kong, Yuling Wang, Changgeng Peng, and Jia-Bao Guo



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Review Article

Effects of Cognitive Behavioral Therapy on Pain and Sleep in Adults with Traumatic Brain Injury: A Systematic Review and Meta-Analysis

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The objective of this study was to systematically review the literature on the effects of cognitive behavioral therapy (CBT) on insomnia and pain in patients with traumatic brain injury (TBI). PubMed, Embase, the Cochrane Library, Cumulative Index to Nursing and Allied Health, and Web of Science databases were searched. Outcomes, including pain, sleep quality, and adverse events, were investigated. Differences were expressed using mean differences (MDs) with 95% confidence intervals (CIs). The statistical analysis was performed using STATA 16.0. Twelve trials with 476 TBI patients were included. The included studies did not indicate a positive effect of CBT on pain. Significant improvements were shown for self-reported sleep quality, reported with the Pittsburgh Self-Reported Sleep Quality Index (MD, -2.30; 95% CI, -3.45 to -1.15; $P < 0.001$) and Insomnia Severity Index (MD, -5.12; 95% CI, -9.69 to -0.55; $P = 0.028$). No major adverse events related to CBT were reported. The underpowered evidence suggested that CBT is effective in the management of sleep quality and pain in TBI adults. Future studies with larger samples are recommended to determine significance. This trial is registered with PROSPERO registration number CRD42019147266.

1. Introduction

Traumatic brain injury (TBI) is a global public health and medical priority with an annual incidence estimated at 200~1967 per 100,000 of the population [1]. The age-standardized prevalence of TBI increased by 8.4% from 1990 to 2016 globally [2, 3], and it became the third leading cause of death and disability [4]. Regardless of the extent, both acute and more chronic consequences that lead to permanent behavioral disabilities and pain associated with most TBIs are due to diffuse axonal injury [5]. Approximately 65% of patients who survive moderate-to-severe TBI subsequently suffer from a wide range of symptoms ranging from physical disabilities (pain, fatigue, etc.) to psychological impairments (hypomnesia, depression, anxiety, etc.) [6], which reduces life expectancy and presents a substantial economic burden to victims, their families, and society as a whole [7].

Pain is reported in over 50% of TBI patients [8], with approximately 20% of TBI patients developing possible

neuropathic pain and sleep problems [9]. In most cases, pain is associated with other post-TBI complaints [10], such as sleep disturbance, which represents a vital interventional target, although sleep disorders and pain are sometimes two independent and separately occurring symptoms of TBI. To enhance interventional efficacy, particularly for TBI patients who have severe pain [11], cognitive behavioral therapies should target both sleep and pain due to the negative effect of pain on sleep quality.

Strong evidence has shown that cognitive behavioral therapy (CBT) is beneficial for the nonbrain-injured population that has cognitive impairments, such as those with anxiety, depression, or intellectual disabilities [12, 13], or for the population with acquired brain injuries, such as those who experienced cerebral vascular accident, anoxia, and neurosurgery [14]. The mechanisms underpinning these improvements appear to be that CBT helps TBI patients understand how to identify and change disturbing thought patterns that have a negative influence on behavior and emotions through a psychotherapeutic approach [15, 16]. Therefore, CBT is an alternative option for patients who suffer from pain and are not suitable for drug therapy. The evidence suggests that CBT, as one of the neuropsychological interventions that combines cognitive and behavioral techniques [17], is the "gold standard" treatment for pain-related symptoms in those with a wide range of musculoskeletal or neurological diseases [18]. However, there has been conjecture that CBT is also effective in post-TBI pain (headache or widespread pain). Moreover, CBT has also been recommended as a first-line treatment for other pain-related dysfunctions (such as sleep disorders and neuropathic pain) [19], although no quantitative meta-analysis has been performed to investigate the effects of CBT on sleep quality in adults with TBI. The present systematic review with meta-analysis is therefore aimed at examining the evidence for the effectiveness of CBT programs on pain and sleep quality in patients with TBI.

2. Methods

2.1. Literature Search and Selection Criteria. This meta-analysis was planned, conducted, and reported in adherence with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement [20]. Using search terms such as "traumatic brain injury", "TBI", "cognitive behavio(u)r therapy∗", "CBT", "pain" and "sleep", we searched PubMed, Embase, the Cochrane Library, Cumulative Index of Nursing and Allied Health Literature (CINAHL), and Web of Science for English-language parallel-group studies reporting the effect of CBT in TBI patients published up to July 2021. The search strategies are shown in Appendix S1. Two reviewers (Peiyuan D and Qing D) independently performed the systematic literature search, detected and deleted all duplicate records, screened the titles, and identified abstracts based on relevance. The full-text articles designated for inclusion were reviewed. In addition, the reference lists of the retrieved articles and available review articles were manually checked to identify additional eligible studies.

Studies were selected for detailed review if they fulfilled the following population, intervention, comparison, outcome, and study design (PICOs) framework: (1) population: TBI participants who had brain damage due to external forces (such as direct impact, rapid acceleration or deceleration, a penetrating injury, or blast waves from an explosion) or a subgroup with TBI whose data could be extracted by the authors, with no restrictions on age, sex, or ethnicity (regular medication use was allowed); (2) intervention: any treatment classified as CBT; (3) comparison: no treatment or non-CBT (including pharmacotherapy); and (4) outcomes: primary outcomes were pain (measured by visual analog scales, the McGill Pain Questionnaire (MPQ), a pain diary, or pressure pain thresholds), sleep quality (assessed by the self-reported Pittsburgh Sleep Quality Index (PSQI), and adverse events associated with CBT, which were reported as the number of participants experiencing any adverse event, number of participants who withdrew because of adverse events, and number of participants experiencing any serious adverse event. Data from randomized controlled trials (RCTs) and case studies were extracted, while only data from RCTs were synthesized.

2.2. Data Extraction and Quality Assessment. Data were extracted by Xin L using a customized data extraction form and independently confirmed by another reviewer (Yuwei F). Detailed information was extracted from each study, including first author, year of publication, study design, number of participants (% women), and demographic and outcome data. Detailed descriptions of the CBT intervention and control group in these RCTs were collected. When the same patients were reported in several publications, we retained only the publication with the largest sample size to avoid duplication of information. Discrepancies were resolved through discussion with a third reviewer (Jianping X) to reach a consensus. The Cochrane risk of bias tool [21] was used to assess the methodological quality of the included studies.

2.3. Statistical Analysis and Data Synthesis. Statistical analysis was performed using STATA, version 16.0. In the quantitative data synthesis section, a random effects model was chosen if two or more trials evaluated the same outcome in comparable groups with the mean difference (MD) and 95% confidence interval (CI) calculated for the summary statistics. If two or more control groups received various treatments in one trial, we combined the data from the control groups using the formula recommended by the Cochrane Handbook for Systematic Reviews of Interventions [21]. The median, interquartile range, and sample size of each trial were obtained to estimate the mean and variance for each study using simple and elementary inequalities and approximations if necessary [22]. The I^2 statistic was calculated to assess heterogeneity among studies, with values < 25% indicating no heterogeneity, 25% to 50% indicating low heterogeneity, 50% to 75% indicating moderate heterogeneity, and >75% indicating high heterogeneity.

The potential publication bias was visually assessed by drawing a funnel plot. Additionally, corresponding authors

were contacted to provide details on unreported data, which was required for our meta-analysis. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system was applied to specify the quality of each outcome by categorizing studies into four levels (high, moderate, low, and very low) by accessing the following factors: study design, study limitations (risk of bias), inconsistency, indirectness of study results, imprecision, and publication bias [23] (shown in Appendix S2).

3. Results

3.1. Study Identification and Selection. The initial electronic search returned a total of 737 records, with 619 unique records identified after duplicates were excluded. A total of 566 titles and abstracts were excluded for various reasons (i.e., they were reviews, letters, or irrelevant to the analysis). Of the remaining 53 articles, 6 RCTs and 6 case studies covering 476 patients were included based on the inclusion criteria. Figure 1 shows the PRISMA flow diagram of the studies in this review.

3.2. Study Characteristics. The demographic and baseline clinical variables of the included studies are shown in Table 1. Studies included both sexes, the mean age of the subjects in the study ranged from 11 to 72 years, and the number of participants in the CBT group ranged from 1 to 200. All studies analyzed in this review included individuals with TBI. Table 2 summarizes the detailed CBT methods in the intervention groups in the RCTs and case studies and the interventional methods in the control groups in the RCTs. Overall, the study duration lasted from 4 weeks to 1 year, with a median of 8 weeks and 4 to 12 sessions. The standard CBT protocol was mentioned in 3 RCTs [24–26] and 1 case study [27], while cognitive behavioral therapy for insomnia (CBT-I) was used in 1 RCT [28] and 4 case studies [29–32]. Two RCTs [33, 34] and 1 case series [35] implemented a CBT-based integrated intervention. An education intervention [26], a wait-list control condition [24, 25], or treatment as usual [28, 33, 34] was conducted in the control groups.

3.3. Quality Assessment. The assessment of the risk of bias for the included RCTs is shown in Table 3. All RCTs reported the numbers and reasons for withdrawal or dropout. Five of the included RCTs generated an adequately randomized sequence [24, 26, 28, 33, 34], and three were conducted in a blinded fashion for the outcome assessment [28, 33, 34]. Given that the pooled number of trials in this comparison was quite small (maximum of 3 trials), no funnel plot analysis was performed.

3.4. Outcome Measurements

3.4.1. Primary Outcomes

(1) Pain. While pain is the main symptom after TBI and has a great impact on quality of life, only 4 RCTs and 3 case studies screened the severity of pain in various forms [24, 25, 33, 34]. Nguyen et al. [34] mentioned that the Brief Pain Inventory data of their participants were obtained at baseline; however, the Brief Pain Inventory was not assessed after the intervention. One of the RCTs used the MPQ [25] to quantify the severity of pain before and after the intervention and found no significant changes after the CBT intervention. Pressure pain thresholds and data from a headache diary were employed as outcome measures in Kjeldgaard and colleague's study, and there was no significant reduction in pain [24]. Moreover, the other RCT [33] used headache pain items from the Traumatic Brain Injury-Quality of Life questionnaire, and no significant improvement in pain was found. Because the three included RCTs [24, 25, 33] had a high degree of heterogeneity in the pain measurements, a meta-analysis of data may have been unconvincing.

In two of the case studies [27, 35], qualitative measures, such as the intensity and frequency of headache and medication use, were recorded at baseline and after a long-term follow-up (from 36 weeks to over one year), and significant improvements were found in the characteristics of the headaches, and much fewer pain killers were used. The Brief Pain Inventory was also used in the study of Lu et al.; however, the effects of CBT on the Brief Pain Inventory scores were contradictory [31].

(2) Sleep Quality. Sleep quality was assessed in 4 RCTs [26, 28, 33, 34] and 4 case studies [29–32]. The PSQI is a self-reported questionnaire that demonstrates good psychometric properties for measuring sleep quality and impairment in various populations [26, 28, 34]. The pooled analysis across three studies [26, 28, 34] showed a significant improvement in self-reported sleep quality in the CBT group (MD, -2.30; 95% CI, -3.45 to -1.15; $P < 0.001$). The heterogeneity among studies was acceptable ($\chi^2 = 0.49$, $P = 0.783$, $I^2 = 0\%$) (Figure 2). The Insomnia Severity Index was used in Nguyen et al.'s [34] and Tomfohr-Madsen et al.'s study [28], and the pooled analysis showed that insomnia was significantly improved in the CBT group (MD, -5.12; 95% CI, -9.69 to -0.55; $P = 0.028$), but the heterogeneity among studies was high ($\chi^2 = 6.31$, $P = 0.012$, $I^2 = 84.2\%$) (Figure 3). Actigraphy, a validated objective test of sleep quality [36] used in Theadom et al.'s study [26], evaluates sleep onset, time awake, and the number of awakenings. However, there were no significant differences in the actigraphy measures after 6 weeks of a CBT-based online intervention. Additionally, in the two RCTs that recruited adolescents [28, 33], positive changes were also found in other sleep quality measures, such as the Dysfunctional Beliefs and Attitudes about Sleep Scale, a sleep diary, and the Adolescent Sleep Wake Scale.

The Insomnia Severity Index was used in all 4 case studies [29–32], and most of the participants showed a negative trend in the Insomnia Severity Index scores, which indicated improvements in insomnia, although the decrease in insomnia severity was not clinically significant in the study of Lu et al. [31]. Sleep diaries were another useful tool for recording daily sleep habits, and quantified data from sleep diaries,

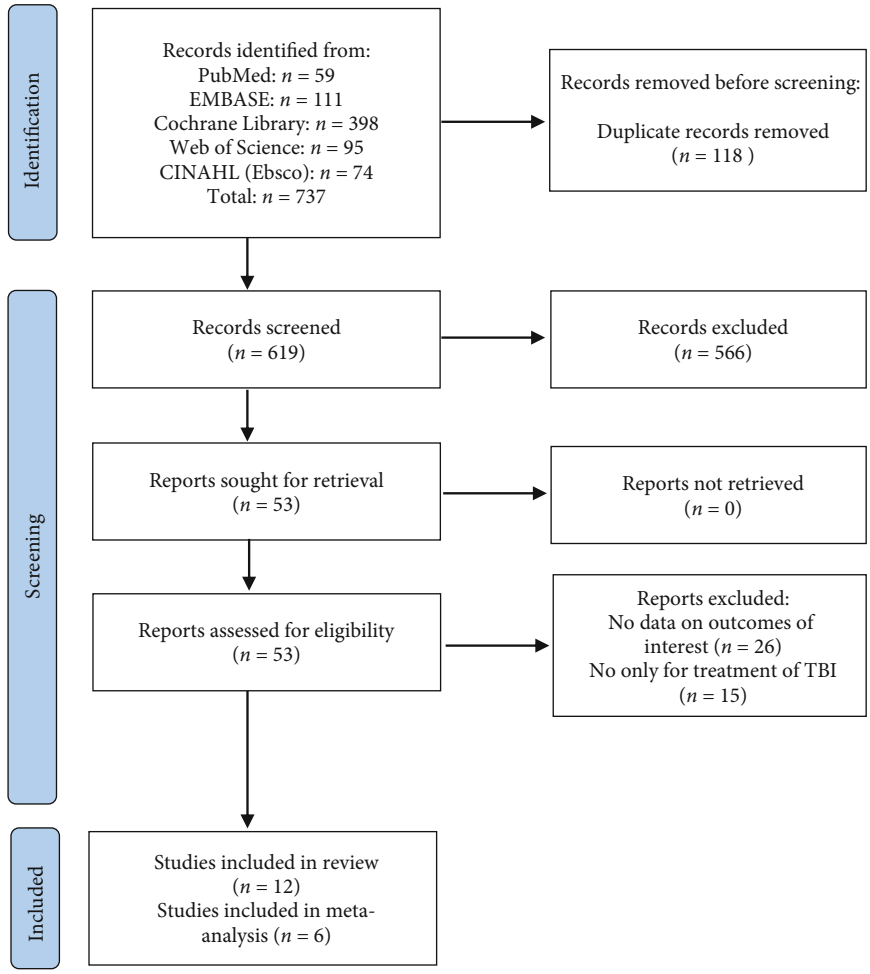

Figure 1: PRISMA flow diagram of studies in this review.

such as total sleep time and sleep efficiency, showed positive changes in the TBI participants with sleep disturbances in the 4 case studies [29–32].

(3) Adverse Events. CBT was well tolerated among the participants in most included studies. An average of 5.3 participants withdrew during the CBT intervention, and the overall dropout rate was 7.8% in the 6 included RCTs, mainly due to loss to follow-up or active withdrawal. Furthermore, no major adverse events, such as progression of symptoms, suicide, or death, were reported among the participants during the CBT intervention.

4. Discussion

The primary purpose of this meta-analysis was to examine the relative efficacy between CBT treatments and non-CBT treatments for TBI. The principal finding of this systematic review and meta-analysis of TBI was that CBT is associated with a significant improvement in self-reported sleep quality but not pain, and CBT was found to be well tolerated among these patients.

Due to the heterogeneity in pain evaluation methods across studies, a meta-analysis could not be performed, although the general trend of the results on pain was described. The TBI patients in the CBT groups did not have significant changes in pain or headache measured by questionnaires or a hand-held pressure algometer after the entire intervention in the included RCTs [24, 25, 33]. Contrary to the expectations that CBT would have marked efficacy on pain, even slight changes could not be discriminated considering the placebo effect of CBT. The reason for the lack of significance may be that most of the included patients suffered from long-term TBI sequelae, and the pain experience in the TBI patients may be profound and chronic. A neuroimaging study showed that chronic pain would remodel sensorimotor activation in the gray matter of the brain, such as widespread alterations in somatosensory cortices, supplementary motor areas, and superior temporal gyri [37]. It is estimated that if CBT or pain education is employed in the early stage after brain trauma, pain symptoms may not enter the chronic stage [24], while pharmacological therapy seems more effective in chronic pain [38]. In contrast to the results from the RCTs, a decrease in the intensity and frequency of headache was found in the two case studies [27, 35]. The mechanisms of the effects of CBT on pain relief lie in changing thoughts as they relate to pain and improving pain through behavioral reinforcement. These improvements

Table 1: Baseline demographic and clinical characteristics of study participants.

	Author (year)	Study type	No. of participants (% women)	Age (y): range/ mean (SD)	Time since injury mean (SD)	Severity	Outcome measures	Adverse events	Time points	Dropout rate after intervention
1	McCarty et al., 2021 [33]	Randomized controlled trial	*T*: 200 (62.0) *I*: 101 (59.4) C:99 (69.6)	*T*: 14.7 (1.7) 11 to 18 *I*: 14.8 (1.7) *C*: 14.7 (1.7)	*I*: 0-30 days: 2 31-60 days: 62 61-90 days: 19 91-120 days: 8 121-180 days: 5 181-270 days: 5 *C*: 0-30 days: 4 31-60 days: 55 61-90 days: 17 91-120 days: 13 121-180 days: 5 181-270 days: 5	*T*: Mild:200	(1) Postconcussive symptoms (the Health Behavior Inventory) (2) Health-related quality of life (the Pediatric Quality of Life Inventory) (3) Psychological assessment: (the Patient Health Questionnaire–9, Generalized Anxiety Disorder-7 item scale, the 15-item anxiety subscale of the Revised Child Anxiety and Depression Scale-Short version) (4) Sleep quality (Adolescent Sleep Wake Scale) (5) Headache pain (Traumatic Brain Injury-Quality of Life headache pain)	None	*I*: baseline; 6-month intervention; 6-month follow-ups *C*: baseline; 6 months; 6-month follow-ups	*I*: 1.98% *C*: 4.04% *T*: 3.00%
2	Tomfohr-Madsen et al., 2019 [28]	Randomized controlled trial	*T*: 24 (75.0) *I*: 12 (75.0) *C*: 12 (75.0)	*T*: 15.0 (1.4) 12 to 18 *I*: 15.2 (1.5) *C*: 14.9 (1.3)	*T*: 1 month to 12 months	*T*: Mild: 24	(1) Sleep disturbance (Insomnia Severity Index, Pittsburgh Sleep Quality Index, Dysfunctional Beliefs and Attitudes about Sleep Scale, sleep diary) (2) Postconcussive symptoms (the Health Behavior Inventory) (3) Psychological assessment (Patient-Reported Outcomes Measurement Information System (PROMIS) Anxiety and Depression)	None	*I*: baseline; 6-week intervention; 4-week follow-ups *C*: baseline; 6 weeks; 4-week follow-ups	*I*: 16.67% *C*: 8.33% *T*: 12.50%
3	Theadom et al., 2018 [26]	Randomized controlled trial	*T*: 24 (62.5) *I*: 12 (58.3) *C*: 12 (66.7)	*T*: 35.9 (11.8) 17 to 56	*I*: 10.42 (7.32) months *C*: 15.09 (10.67) months	*I*: Mild: 11 Moderate: 1 *C*: Mild: 11 Moderate: 1	(1) Sleep disturbance (Pittsburgh Sleep Quality Index, actigraphy sleep onset) (2) Cognitive function: (CNS vital signs online neuropsychological assessment) (3) Quality of life: (Quality of Life after Brain Injury questionnaire)	None	*I*: baseline; 6-week intervention *C*: baseline; 6-week intervention	*I*: 16.67% *C*: 16.67% *T*: 16.67%

Table 1: Continued.

	Author (year)	Study type	No. of participants (% women)	Age (y): range/ mean (SD)	Time since injury mean (SD)	Severity	Outcome measures	Adverse events	Time points	Dropout rate after intervention
4	Nguyen et al., 2017 [34]	Randomized controlled trial	*T*: 24 (33.3) *I*: 13 (30.8) *C*: 11 (36.4)	*I*: 45.53 (13.87) *C*: 41.90 (12.95) *T*: 43.87 (12.95)	77 days to 20.47 years) *I*: 759.15 (714.23) days *C*: 2093.36 (2192.62) days	*I*: Mild: 4 Moderate: 1 Severe: 8 *C*: Mild: 1 Moderate: 1 Severe: 9	(1) Sleep disturbance (Pittsburgh Sleep Quality Index, Insomnia Severity, Index, Epworth Sleepiness Scale) (2) Fatigue: (Brief Fatigue Inventory, Fatigue Severity Scale) (3) Psychological assessment: (Hospital Anxiety and Depression Scale, anxiety and depression)	None	*I*: baseline 2-month intervention; 2-month follow-ups *C*: baseline; 2 months; 2-month follow-ups	*I*: 0% *C*: 0% *T*: 0%
5	Potter et al., 2016 [25]	Randomized controlled trial	*T*: 46 (45.7) *I*: 26 (42.3) *C*: 20 (50.0)	*I*: 40.1 (10.3) *C*: 43.1 (13.1) *T*: 41.4 (11.6)	*I*: 6–12 months: 6 >12, ≤24 months: 6 >24 months: 14 *C*: 6–12 months: 7 >12, ≤24 months: 3 >24 months: 10	*I*: Mild: 12 Moderate: 7 Severe: 6 *C*: Mild: 6 Moderate: 5 Severe: 14	(1) TBI symptom (Rivermead Post-Concussion Symptoms Questionnaire, Brain Injury Community Rehabilitation Outcome Scale, Impact of Event Scale) (2) Psychological assessment: (Hospital Anxiety and Depression Scale, Anxiety and Depression State-Trait Anger Expression Inventory-2) (3) Pain and fatigue (McGill Pain Questionnaire, Checklist of Individual Strength) (4) Quality of life: (Quality of Life Assessment Schedule, European Quality of Life)	None	*I*: baseline 12-week intervention *C*: baseline; 12-week intervention	*I*: 3.85% *C*: 0% *T*: 2.17%
6	Kjeldgaard et al., 2014 [24]	Randomized controlled trial	*T*: 90 (55.6) *I*: 45 *C*: 45	*T*: 34 (11.3)	Not mentioned	*T*: Mild: 90	(1) Pain and headache (basic headache diary, pressure pain thresholds) (2) TBI symptom (Rivermead Post-Concussion Symptoms Questionnaire) (3) Psychological assessment (Symptom Checklist) (4) Quality of life (36-item Short Form Health Survey)	None	*I*: baseline; 16-week intervention *C*: baseline; 16-week intervention	*I*: 22.22% *C*: 17.78% *T*: 20.00%
7	Lah et al., 2019 [32]	Case report	*T*: 5 (20.0)	*T*: 11.8 (0.8)	*T*: 7.4 (2.9) years	Moderate: 2 Severe: 3	(1) Sleep quality (sleep diaries, actigraphy watches, Insomnia Severity Index, Pittsburgh Sleep Quality Index) (2) Fatigue (PedsQL Multidimensional Fatigue Scale)	None	Baseline 4-week intervention 1-week follow-up	After intervention *T*: 28.57% After follow-up *T*: 42.86%

Table 1: Continued.

	Author (year)	Study type	No. of participants (% women)	Age (y): range/ mean (SD)	Time since injury mean (SD)	Severity	Outcome measures	Adverse events	Time points	Dropout rate after intervention
8	Baker et al., 2018 [35]	Case report	*T*: 25 (32.0)	*T*: 29	*T*: 1 month-10 years (average 26 months)	Mild: 25	(1) Pain and headache (migraine frequency, duration, and severity) (2) Quality of life (3) Occupational assessment (current deployment and duty status)	None	Baseline 2-year follow-up	*T*: 0%
9	Lu et al., 2016 [31]	Case report	*T*: 3 (66.7)	*T*: 53.7 (10.1) Case 1: 60 Case 2: 42 Case 3: 59	Case 1: 6 years Case 2: 2 years Case 3: 1 year	Case 1: Mild Case 2: Moderate Case 3: Severe	1. Sleep quality (Insomnia Severity Index, Pittsburgh Sleep Quality Index, Dysfunctional Beliefs and Attitudes about Sleep Scale–Brief Version) 2. Pain (Brief Pain Inventory) (3) Psychological assessment (the Hospital Anxiety and Depression Scale (HADS): anxiety; depression) (4) Fatigue (Multidimensional Assessment of Fatigue-Global Fatigue Index)	None	Baseline 4-week intervention 1-3-month follow-up	*T*: 0%
10	Ouellet and Morin, 2007 [29]	Case report	*T*: 11 (45.5)	*T*: 27.3 (8.5)	*T*: 27.5 (9.7) months	Mild: 1 Mild-moderate: 2 Moderate: 2 Moderate-severe: 3 Severe: 3	(1) Sleep quality (sleep diary, Insomnia Severity Index, Dysfunctional Beliefs and Attitudes about Sleep Scale) (2) Fatigue (Multidimensional Fatigue Inventory) (3) Psychological assessment (Beck Depression/Anxiety Inventory)	None	Baseline 8-10-week intervention 1-3-month follow-up	*T*: 0%
11	Gurr and Coetzer, 2005 [27]	Case report	*T*: 41 (31.7)	*T*: 44.05 Range: 22-78	*T*: 78.7 (108.3) months	Mild: 18 Moderate: 7 Severe: 16	(1) Pain and headache (Headache Disability Inventory, Headache Needs Assessment, Chronic Pain Index) (2) Quality of life (Nottingham Health Profile) (3) Psychological assessment (Hospital Anxiety and Depression Scale)	None	Baseline 14-15-week interventions 12-13-week follow-up	*T*: 51.2%
12	Ouellet and Morin, 2004 [30]	Single-case study	*T*: 1 (0)	*T*: late thirties	1 year	Moderate	(1) Sleep disturbance (sleep diary, polysomnography data, Insomnia Severity Index, Dysfunctional Beliefs and Attitudes about Sleep Scale) (2) Psychological assessment (Beck Anxiety Inventory, Beck Depression Inventory)	None	Baseline 8 weeks of CBT 1-month follow-up 3-month follow-up	*T*: 0

Table 2: Cognitive behavioral therapy and control interventions in the included parallel-group trials.

	Author (year)	Cognitive behavioral therapy in the intervention group	Control group intervention	Frequency	Duration
1	McCarty et al., 2021 [33]	Hybrid (telehealth and face-to-face) individualized intervention with care management and enhanced medication consultation	Usual health care	1 hour per week	6 months
2	Tomfohr-Madsen et al., 2019 [28]	Insomnia-specified individualized intervention	Usual health care	45 minutes per week	6 weeks
3	Theadom et al., 2018 [26]	Online individualized intervention with interactive features or suggestions on behavior change	Online education without interactive features or suggestions on behavior change	20 minutes per week	6 weeks
4	Nguyen et al., 2017 [34]	Face-to-face individualized intervention with 30-minute exercise	Usual health care	Moderate exercise 30 minutes 3 to 5 times per week & cognitive behavioral therapy 1 session per week	2 months
5	Potter et al., 2016 [25]	Face-to-face individualized intervention	Waiting list control	1 hour per week	12 weeks
6	Kjeldgaard et al., 2014 [24]	Face-to-face group intervention	Waiting list control	2 hours per week	9 weeks
7	Lah et al., 2019 [32]	Face-to-face insomnia-specified individualized intervention	/	75 minutes per week	4 weeks
8	Baker et al. 2018 [35]	Face-to-face individualized intervention with lifestyle modifications	/	Not mention	2 years
9	Lu et al., 2016 [31]	Insomnia-specified individualized intervention	/	1 hour per week	4 weeks
10	Ouellet and Morin, 2007 [29]	Face-to-face insomnia-specified individualized intervention	/	1 hour per week	8-9 weeks
11	Gurr and Coetzer, 2005 [27]	Face-to-face group relaxation & face-to-face individualized therapy session	/	Group intervention per week for 3 weeks & individualized intervention 30 mins per two weeks for 12 weeks	14-15 weeks
12	Ouellet and Morin, 2004 [30]	Face-to-face insomnia-specified individualized intervention	/	1 session per week	8 weeks

require long-term CBT treatment. The CBT interventions in the case studies lasted for a long time, and the main intervention target was headache, so there was a positive intervention effect. Sleep disorders, which might be associated with diffuse axonal injury resulting in damage to sleep-regulating structures and disruptions in hypocretin-1, can be categorized: insomnia was found in 29%, hypersomnia in 28%, and sleep apnea in 25% of patients who have a history of TBI [39]. Sleep has a significant impact on the quality of life of TBI patients, and sleep disturbances have been consistently related to anxiety, depression, fatigue, or other complications. Many studies have reported that PSQI scores are associated with subjective questionnaire scores for anxiety and depression [40–42]. Although evidence on CBT specific to patients with TBI was scarce, our meta-analysis found a significant improvement in self-reported sleep quality measured with the PSQI, which was in accordance with the results of Ouellet MC's study [43]. However, there were no significant changes in actigraphy measures. Sleep quality is more like a subjective experience, and CBT could subjectively change participants' thoughts in relation to sleep and improve sleep behavior. As a result, self-reported sleep quality rose, and the objective data (actigraphy measures) may not improve as much as the subjective measures. Greater heterogeneity appeared in the data synthesis of the Insomnia Severity Index. In the two included RCTs, there were great difference characteristics of the participants, as female adolescents accounted for 75% of the participants in Tomfohr-Madsen et al.'s study [28], while the age span of the participants in Nguyen et al.'s study [34] was large. However, the biggest contributor to the heterogeneity was from the difference in baseline symptoms of insomnia. Insomnia in the participants in Tomfohr Madsen et al.'s study [28] was more severe than that in Nguyen et al.'s study [34], and CBT is known to achieve larger effect sizes in groups with more severe insomnia. To a certain extent, our results were partially contrary to Ford et al.'s conclusion that there was a reliable effect in support of CBT for TBI patients with sleep disorders [44]. Several methodological differences may be proposed to explain the contrasting findings. Whereas

Table 3: The Cochrane Collaboration's tool for assessing risk of bias for methodological assessment.

Article (year)	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessments	Incomplete outcome data	Selective reporting	Other bias
McCarty et al., 2021	Low	Unclear	High	Low	Low	High	Unclear
Tomfohr-Madsen et al., 2019	Low	Unclear	High	Low	High	Low	Unclear
Theadom et al., 2017 [26]	Low	Low	Unclear	Unclear	High	Low	Unclear
Nguyen et al., 2017 [34]	Low	Unclear	High	Low	Low	Low	Unclear
Potter et al., 2016 [25]	Unclear	Unclear	High	High	Low	High	Unclear
Kjeldgaard et al., 2014 [24]	Low	Low	High	High	High	High	Unclear

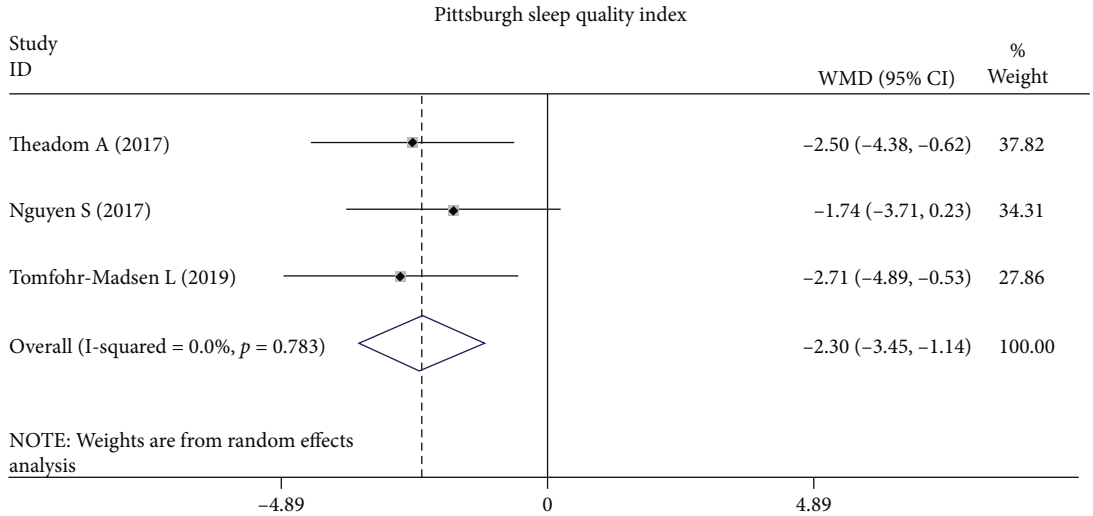

Figure 2: Differences in Pittsburgh Self-Reported Sleep Quality Index scores following CBT compared with other forms of interventions.

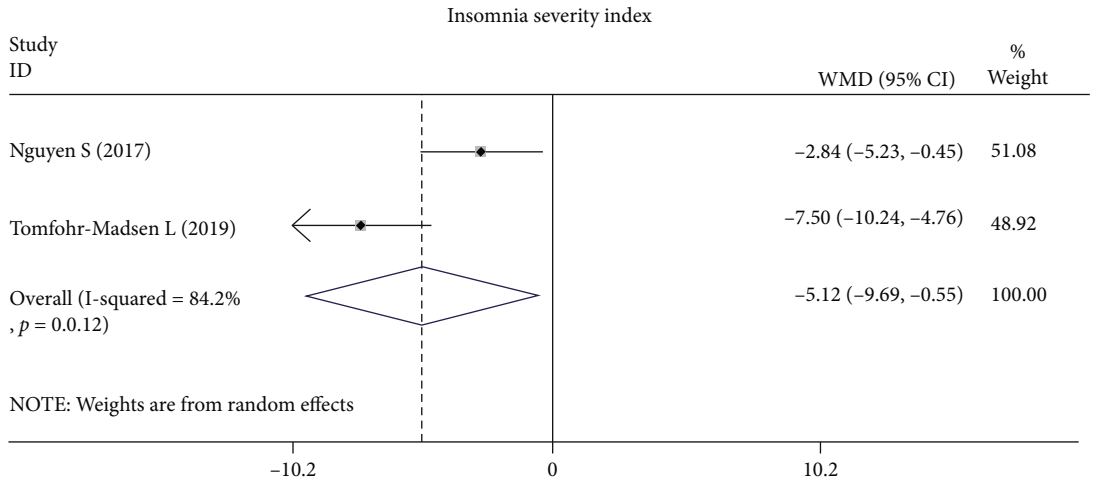

Figure 3: Differences in Insomnia Severity Index scores following CBT compared with other forms of interventions.

Ford and colleagues included 7 trials, comprising both clinical trials and single case studies, the present meta-analysis included only RCTs and was more concentrated on CBT and TBI. Last but not least, this was the first meta-analysis that synthesized evidence using quantitative methods, which provided a more objective estimate of the treatment effects.

5. Strengths and Limitations

Although CBT is recommended for treating pain and sleep disorders in people after TBI, there has been no systematic review that revealed the therapeutic effects of CBT. This systematic review and meta-analysis is the first to show the therapeutic effect of CBT on posttraumatic pain, especially headache. Sleep quality and insomnia symptoms were also significantly improved. However, there are several limitations in this study. First, we had only a limited number of clinical trials that assessed the efficacy and safety of CBT among patients with TBI; thus, publication bias cannot be completely ruled out. Second, only half of the included studies evaluated quality of life, and none of the included studies assessed TBI-related restrictions to participation in daily life. Third, as the included studies reported outcomes by various methods, it was relatively difficult to derive a powerful synthesis of data evaluating CBT in groups of patients with TBI. Finally, although the meta-analysis showed that there were significant changes in sleep quality and insomnia, the clinical importance of the changes may be limited. Future multicenter, well-designed, large, population-based randomized control trials are needed.

6. Conclusions

CBT is relatively safe and is associated with significant improvements in self-reported sleep quality among patients with TBI, while limited evidence has shown that pain cannot be significantly improved by CBT. Nevertheless, interpretation of our results must be done cautiously considering the methodological drawbacks and poor quality of the data in the included trials. Future studies with more homogeneous, objective assessments are needed to determine whether CBT can be used to improve long-term clinical endpoints among these patients in the real world.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yuwei Feng and Jianping Xia contributed equally to this work as co-first authors.

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Supplementary Materials

Appendix S1: the search strategies for the databases. Appendix S2: results of GRADE criteria. *(Supplementary Materials)*

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Review Article

Spinal Cord Stimulation and Treatment of Peripheral or Central Neuropathic Pain: Mechanisms and Clinical Application

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Spinal cord stimulation (SCS) as an evidence-based interventional treatment has been used and approved for clinical use in a variety of pathological states including peripheral neuropathic pain; however, until now, it has not been used for the treatment of spinal cord injury- (SCI-) induced central neuropathic pain. This paper reviews the underlying mechanisms of SCS-induced analgesia and its clinical application in the management of peripheral and central neuropathic pain. Evidence from recent research publications indicates that nociceptive processing at peripheral and central sensory systems is thought to be modulated by SCS through (i) inhibition of the ascending nociceptive transmission by the release of analgesic neurotransmitters such as GABA and endocannabinoids at the spinal dorsal horn; (ii) facilitation of the descending inhibition by release of noradrenalin, dopamine, and serotonin acting on their receptors in the spinal cord; and (iii) activation of a variety of supraspinal brain areas related to pain perception and emotion. These insights into the mechanisms have resulted in the clinically approved use of SCS in peripheral neuropathic pain states like Complex Regional Pain Syndrome (CRPS) and Failed Back Surgery Syndrome (FBSS). However, the mechanisms underlying SCS-induced pain relief in central neuropathic pain are only partly understood, and more research is needed before this therapy can be implemented in SCI patients with central neuropathic pain.

1. Introduction

Electrostimulation for pain therapy emerged in the convergence of Pacemaker technology, the "Gate control" theory of pain, and pioneering clinical trials from 1950s to 1960s [1, 2]. According to this theory, the activation of low-threshold nonnociceptive fibers closes the gate of the nociceptive signal input through the activation of inhibitory neurons in the spinal cord to suppress pain [1]. SCS is a form of electrotherapy by implanting electrodes into the epidural space in the spinal cord and stimulating the dorsal column to modulate neural function. The first-generation of SCS devices was comprised of two main components: an electrode and a pulse generator. The original electrode was a design based on Torresani et al.'s cardiac pacemaker that incorporated twisted platinum tinsel wire in a Dacron filament matrix [3]. SCS was first successfully used in 1967 by Shealy et al. for the treatment of pain in patients [4]. Nowadays SCS is still used for chronic pain treatment with modified leads, advanced remote pulse generators, and various stimulation parameters/programs such as conventional SCS (i.e., tonic stimulation, at a frequency of 30-80 Hz, 100 to 500 μs of pulse width, and an amplitude above sensory threshold), high-frequency stimulation (at a frequency of 1-10 kHz, with a pulse width at approximately 30 μs, and an amplitude of typically 1 to 5 mA), high-frequency burst stimulation (at a frequency of 40 Hz with 5 closely spaced pulses at 500 Hz per burst), and dorsal root ganglion

stimulation [5–7]. Low-frequency SCS has been suggested to be better for treatment of heat hyperalgesia due to C-fiber neuropathy, while high-frequency SCS may be better for modulation of mechanical allodynia due to A-fiber neuropathy [8]. More recently, high-frequency simulation (500 Hz) as compared to low-frequency (5 Hz) and conventional stimulation has been shown to induce a delayed effect on mechanical allodynia in an animal model of painful diabetic polyneuropathy [9]. However, some limitations of SCS have been reported during clinical applications including equipment-associated limitations, such as contamination of implanted leads or pulse generator, the pain caused by the pressure of implanted leads on the nervous system, or discomfort caused by the implanted pulse generator device [10–12]. Current investigations are trying to balance between the advantages and disadvantages of the SCS technique.

Conventional SCS directly stimulates the large diameter nonnociceptive Aβ-fibers in the dorsal column, and then it antidromically inhibits those nociceptive signals which enter the spinal dorsal horn. The electrical pulses generated by the stimulator propagate not only antidromically but also orthodromically along the nonnociceptive nerve fibres. Although it was known that the activation of "Gate control" is attributed to antidromic stimulation, the effects of orthodromic stimulation were largely unknown at that time. In the following two decades, the mechanisms of SCS-induced analgesic effects at the supraspinal level were gradually unraveled. It was found that SCS can modulate pain perception by the activation of some supraspinal pain processing systems such as the thalamic centrum medianum and the pretectal nucleus [13, 14]. Since the discovery of the descending bulbospinal pathways [15], accumulating evidence has shown that the nociceptive-evoked activity in a number of supraspinal areas which are related to pain transmission like the locus coeruleus (LC), the rostral ventromedial medulla (RVM), the reticular formation (RF), and the periaqueductal gray (PAG) can be inhibited by electrical stimulation [16, 17]. From then on, a growing number of studies focused on the alterations in inhibitory neurotransmitters including GABA, serotonin, acetylcholine, opioids, and endocannabinoids in response to SCS, indicating that the "spino-bulbospinal" loop was tuned by SCS [16, 18, 19]. Since chronic pain always contains emotional, motivational, and cognitive components which are manifested as mood disorders, the investigation on the influence of SCS on these aspects of pain might facilitate the understanding of the mechanisms of SCS-induced analgesia [20]. While a growing number of studies showed that the motor system and the sympathetic system could be modulated by SCS to improve the locomotor function after SCI and alleviate angina pectoris [21, 22], in this review, we focus on the effects of conventional SCS on neuromodulation of peripheral and spinal cord injury-induced central neuropathic pain.

2. Mechanisms of Peripheral and Central Neuropathic Pain

Neuropathic pain (NP) is a complex, heterogeneous disorder that affects approximately 8% of the total adult human population and comes with significant burden for both the patient and the healthcare system [23]. The International Association for the Study of Pain (IASP) defines NP as follows: "pain that arises as a direct consequence of a lesion or disease affecting the somatosensory system" [24]. The origin of NP might be either due to nerve injury of peripheral nerves (peripheral neuropathic pain (PNP)) or due to a central nerve injury (central neuropathic pain (CNP)). PNP is common in CRPS, FBSS, and some diseases resulting in peripheral nerve damage like cancer and diabetes, whereas the CNP usually occurs after stroke, spinal cord injury, or multiple sclerosis [25]. NP is characterized by spontaneous pain (which happens spontaneously without stimuli like burning and tingling etc.), allodynia (response to innocuous stimuli), and hyperalgesia (increased response to noxious stimuli).

During PNP, peripheral tissue injury results in the release of inflammatory mediators/cytokines/chemokines (e.g., PGE2, 5-HT, IL-1β, TGF-β, and chemokine (C-C motif) ligand 2 (CCL2)) and neurotrophic factors (e.g., nerve growth factor) that sensitize nociceptors, leading to altered expression and activity of ion channels in sensory neurons, consequently reducing the mechanical and thermal threshold of nociceptors (peripheral sensitization) [26]; even light-touch mechanoreceptors (e.g., $TrkB^+$ fibers), which do not transduct pain signals in physiological state, start to produce allodynia during PNP [27]. The aberrant excited peripheral neurons release massive amounts of neurotransmitters including glutamate and substance P from the central terminals in the dorsal horn of the spinal cord, and this results in activation of AMPA/NMDA and NK receptors, respectively, inducing long-lasting increased excitability of dorsal horn neurons (a process termed central sensitization) [28]. A recent study demonstrated that increased expression of voltage-gated sodium channels like $Na_v1.7$ and $Na_v1.8$ in spinal interneurons is also involved in central sensitization [29]. In addition, the inflammatory mediators released from injured neurons can trigger the activation of microglia, astrocytes, oligodendrocytes, mast cells, and T-cells which in turn release more pronociceptive factors (IL-6, IL-1β, and TNF-α) contributing to the development and maintenance of PNP [30]. Descending inhibition systems are involved in pain modulation during PNP [31]. Due to thoracic spinal cord injury in animal pain models, CNP has "above-level" pain (forelimbs), "at-level" pain (trunk), and "below-level" pain (hindlimbs) [32]. The mechanisms of PNP and CNP have many similarities and some differences. PNP and CNP have in common, as a major similarity, the sensitization phenomenon; however, they differ for the injured location and contributions of sensitization. For example, in CNP, peripheral sensitization is only observed in the "above-level" pain state by "retrograde activation" of peripheral neurons [33], and central sensitization contributes to "at-level" pain and "below-level" pain. Additionally, activated microglia can release PGE2 to modulate the pain processing in dorsal horn neurons in "below-level" pain [34]. The treatment of both PNP and CNP is an unmet need for now since the underlying mechanisms are extremely complicated and have not been fully elucidated.

3. Analgesic Mechanisms of Conventional SCS

The mechanisms of action of SCS were initially modeled with the "Gate control" theory; nonetheless, recent studies have demonstrated the involvement of endocannabinoids, endogenous opioids, and of the descending pain inhibitory systems in the SCS process (Figure 1).

3.1. Segmental Inhibition via GABA, Endocannabinoids, and Endogenous Opioids. Segmental inhibition is implemented via activation of GABAergic inhibitory interneurons in the spinal cord and contributes to the SCS-induced analgesia. The earliest evidence of spinal inhibition was discovered by Lidierth and Wall who suggested that dorsal column stimulation might inhibit afferent discharge [35]. However, the best-known mechanism of segmental inhibition is the "Gate control" theory. Based on this theory, the electrical stimulation of large myelinated Aβ fibers, located in the dorsal columns, results in antidromic stimulation of the nociceptive network in the spinal dorsal horn. Indeed, the GABA release from GABAergic inhibitory interneurons in the spinal cord was increased after SCS treatment in animals [18, 36]. The increased GABA activates $GABA_A$ receptors on the presynaptic neurons to inhibit the excitatory neurotransmission between glutamatergic nociceptive C-fibers and the wide dynamic range (WDR) neurons in the spinal dorsal horn [36, 37]. A clinical study found that excitability of spinal dorsal horn neurons, particularly WDR neurons, might be inhibited by SCS in chronic neuropathic pain patients [38].

Nevertheless, there is increasing evidence for other mechanisms involved in SCS-induced pain modulation within the spinal "Gate control." Recently, it has been reported that endocannabinoid activation of cannabinoid receptor 1 (CB_1R) contributes to long-lasting reversal of neuropathic pain by repetitive SCS to the dorsal columns in rats [39]. It has been demonstrated that SCS can prime the nervous system to evoke more analgesia over time, and this is persistent for several days [39]. Another study consistently reported that blockade of CB_1R in both excitatory and inhibitory neurons in superficial dorsal horns of the spinal cord attenuated postsynaptic currents caused by electrical stimulation of Aβ fibers [40]. Previous studies carried out in the last 25 years proved that there were two CB_1R endogenous ligands, N-arachidonoyl-ethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG) [41, 42]. Since their discovery as high (anandamide) and low-to-moderate (2-AG) affinity ligands for CB_1Rs, it also became clear that the two major endocannabinoids exhibit varying efficacy as CB_1R agonists [41, 42]. Since CB_1R is localized preferentially in brain areas involved in pain transmission, such as the cortex, PAG, and in the spinal cord [43, 44], SCS may exert antinociception through activating CB_1Rs in these areas via both orthodromical and antidromical stimulation.

Moreover, several kinds of other endogenous neurotransmitters including opioids and acetylcholine have been proven to be underlying SCS mechanisms for pain relief [45, 46]. The opioid receptors are involved in the pain relief induced by SCS in a frequency-dependent manner since both 4 Hz and 60 Hz SCS work through opioid receptor mechanisms, with 4 Hz SCS activating μ-opioid receptors, while 60 Hz SCS-activated δ-opioid receptors [45]. Furthermore, SCS was shown to attenuate peripheral neuropathic pain via activation of the cholinergic system through muscarinic receptor 4, but not through nicotinic receptors in rats [46]. These studies proved that endogenous analgesics or inhibitory mediators are involved in SCS-induced analgesia.

Apart from the alteration of neurotransmitters released in the spinal cord, SCS also reversed the increased pain-related genes which code for proinflammatory cytokines like IL-1 and IL-6 in the dorsal root ganglion in a spared nerve injury model [47], pointing out its role in the peripheral nervous system. Moreover, the release of proinflammatory cytokines by spinal glial cells might be indirectly modulated by SCS. It is known that astrogliosis occurs and microglial GluN2B increases after SCI in rats [48], and the local astroglial scar is proven to hamper the neuroregeneration in the injured spinal cord. Additionally, the proinflammatory cytokines and chemokines released from activated microglia due to SCI contribute to the central sensitization in neuropathic pain. Sato et al. reported that SCS significantly reduced the immunostaining density of marker proteins of astrocytes and microglia bilaterally in rat spinal cord 2 weeks after peripheral nerve injury [49] and SCS attenuated neuropathic pain by suppression of spinal glial activation [50]. The information indicates the possible role of SCS in reducing astrogliosis in spinal-cord-injured rats.

3.2. Stimulation-Induced Descending Inhibition. Based on the understanding of descending pain control at the supraspinal level [51, 52] and the orthodromic effect of SCS, SCS might alter the responses of supraspinal systems upon the incoming nociceptive signals in the spinal cord by modulating the balance of descending facilitation and inhibition. A clinical study indirectly proved the role of SCS at the spinal/supraspinal level by increased sensory threshold in both pain areas and nonpain areas of chronic pain patients [53]. It indicates that electrical stimulation may not only activate large myelinated fibers in the dorsal column but also have an influence on the ascending or descending tracts in the ventrolateral column. In agreement, an increasing number of studies demonstrated that descending inhibition was evoked by SCS leading to the release of neurotransmitters including noradrenalin and serotonin which modulate WDR neurons in the spinal cord, and thus playing an important role in the antinociceptive mode of action of SCS.

Moreover, it has been shown that electrical stimulation of the descending fibers originating in certain brainstem areas such as LC and RVM can inhibit the input of nociceptive signals into the brain [54]. Furthermore, electrical stimulation of the A6-A7 nuclei in LC, known to be the source of spinally projecting noradrenergic neurons, inhibits the hypersensitivity in the spinal dorsal horn following noxious challenge [55]. A recent study showed that SCS increased the neuron activity in LC in a peripheral neuropathic pain model but did not change the expression of noradrenaline in the spinal dorsal horn compared to that without SCS [56]. These studies confirmed that the LC neurons are

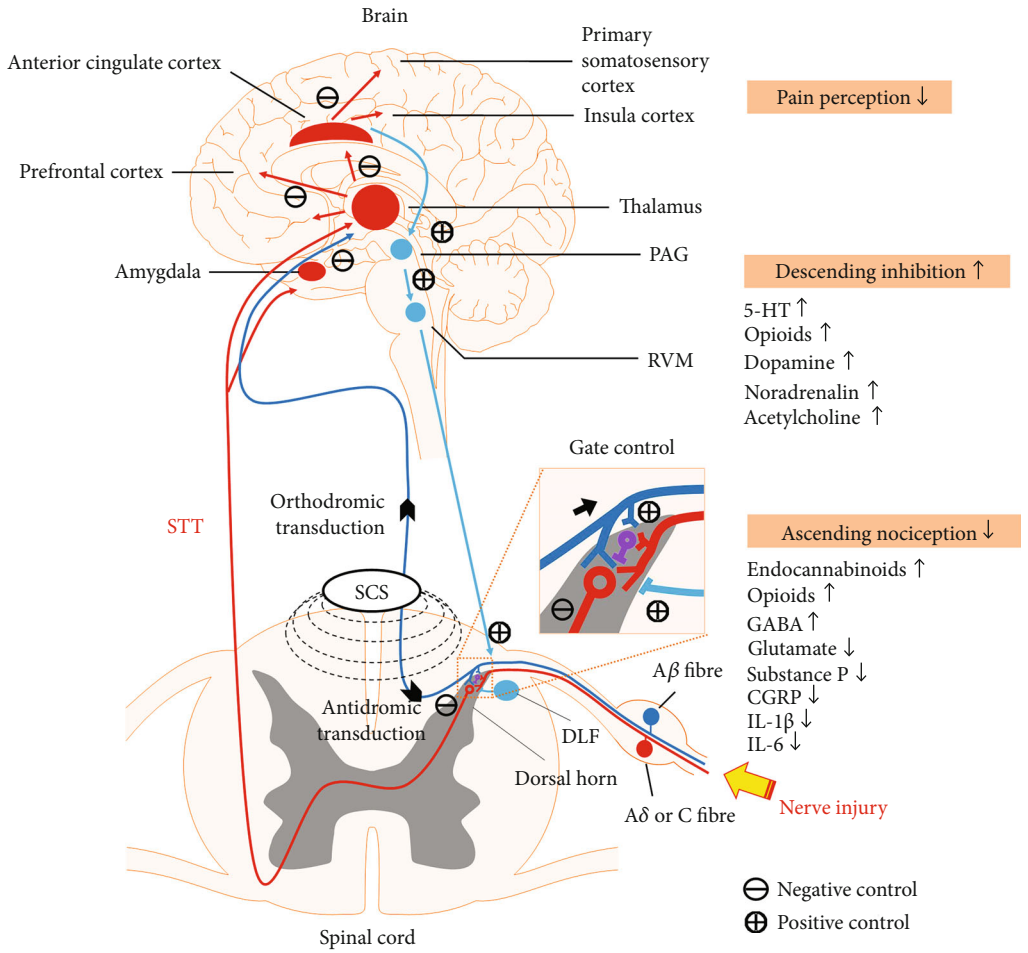

Figure 1: Schematic drawing shows the effects of spinal cord stimulation on nociceptive processing including segmental spinal inhibition, activation of descending inhibitory system, and cortical modulation. SCS: spinal cord stimulation; STT: spinothalamic tract; PAG: periaqueductal gray; RVM: ventrolateral medulla; DLF: dorsolateral funiculus.

indeed activated by SCS; however, their roles in SCS-induced antinociception needs to be further elucidated.

In contrast to the undefined effects of the noradrenergic system, the role of SCS in the modulation of descending serotoninergic fibers originating from the RVM has been investigated in much more detail. It has been found that the release of 5-HT in the spinal laminae I-II was increased in SCS-treated rats with neuropathic pain [8]. Meanwhile, the immediate early gene c-Fos expression in the RVM was shown to be increased after SCS [9]. Moreover, SCS-induced inhibition of the mechanical hypersensitivity due to peripheral nerve injury could be blocked by intrathecal injection of antagonists of selected serotonergic receptors 5-HT_{2A} and 5-HT_4 [19]. Recently, it was reported that the dysfunction of descending inhibitory serotonergic neurons in the RVM by microinjection of a $GABA_A$ receptor agonist, attenuated the SCS-induced inhibition of nociceptive processing [57]. Although the diverse cell types in the RVM exert different influences at the dorsal horn to facilitate or inhibit nociceptive signal transmission, SCS might tip the balance in favor of inhibition.

PAG is a major nucleus within the midbrain involved in pain inhibition, and it sends its projections via the RVM to the spinal dorsal horn [58]. The descending inhibition of neuronal responses to noxious thermal stimulation is induced by bipolar focal electrical stimulation in PAG [59]. Additionally, 100 Hz SCS with a current of two-thirds of the motor threshold for 30 min and a repeated SCS at 2 hours decrease extracellular concentrations of GABA in ventrolateral PAG in free-moving rats without nerve injury [18]. However, a nonsignificant activation of neurons was noted in the ventrolateral and dorsolateral PAG of spared nerve injured (SNI) rats in response to 100 Hz SCS with a current of 80% of the motor threshold [9]. The discrepancy may occur due to the different use of stimulation parameters as mentioned above and the alteration of nociceptive pathways after SNI. The involvement of PAG in the descending analgesia induced by SCS needs to be confirmed by further studies.

Since the dorsolateral funiculus (DLF) is part of the descending pain inhibitory pathway [60], DLF lesions attenuated the suppressive effect of SCS on thermal hyperalgesia and mechanical allodynia by about 50% in a peripheral neuropathic pain model [61]. Moreover, with the use of two sets of electrodes rostrally at dorsal column nuclei (DCN) and SCS at lower thoracic levels with a DLF lesion at the cervical

level in between, the effect of DCN stimulation was equal to that produced by SCS without a DLF lesion, providing further evidence for the involvement of supraspinal control in the mode of action of SCS. With central neuropathic pain due to SCI in patients, the DLF is often also injured and thus the descending inhibitory control is limited. SCS can no longer act via this supraspinal route, and thus, part of the SCS-induced analgesic effect is diminished in the SCI-induced CNP state. In a unilateral dorsal quadrant lesion SCI model in rats, Sun et al. revealed that the DLF lesion not only blocked the majority of the analgesic effect of SCS but also decreased the activation of neural progenitors evoked by SCS 2 weeks after SCI [62]. In conclusion, descending inhibitory systems originating in the brainstem play an essential role in SCS-induced suppression of peripheral and central neuropathic pain.

3.3. Modulation of Pain Perception. Dorsal column SCS may have neuromodulatory effects at cortical levels, although understanding the mechanism is extremely complex and far from being completely understood. A recent fMRI study using the partial sciatic nerve ligation-induced PNP model in rats demonstrated that the higher centers of the pain perception system comprising the thalamus, somatosensory cortex, insular cortex, anterior cingulate cortex, limbic network, hippocampus, and nucleus accumbens were tuned by conventional SCS via interactions in multiple pain pathways, and even more brain areas like the raphe nuclei and caudate putamen were activated by an active recharge burst SCS [63]. These massive SCS-evoked brain areas are not only pain related but also cognition/motivation related, suggesting that SCS cannot only reverse the lowered mechanical threshold due to nerve injury but also improve the cognitive-motivational aspects of pain [64]. An experimental study showed that dorsal column stimulation evoked negative responses in somatosensory cortex (SS) I, SSII, and thalamic nuclei in monkeys [65]. In a rat model, expression of c-Fos was increased after SCS treatment in nuclei of the thalamus and forebrain including the insular cortex and amygdala that are involved in the processing of pain [66]. Moreover, a clinical study demonstrated that activation of SSI, SSII, and cingulate regions were detected by functional magnetic resonance imaging (fMRI) in patients with significantly successful pain treatment of SCS [67]. The anterior cingulate cortex was activated by SCS in patients with low limb and back pain to reduce patients' attention to pain [68]. More recently, a cortical function assessment using fMRI elucidated that the cortical connectivity between the somatosensory cortex and limbic areas was decreased by SCS in pain patients with peripheral neuropathic pain (CRPS) [69]. SCS can alleviate not only pain but also anxiety and depression in FBSS patients [70]. Additionally, SCS-induced pain relief is associated with a significant reduction of anxiety, catastrophizing, and disability [71]. It indicated that SCS of the dorsal column may modulate pain perception by reducing negative emotional processing of pain. However, a better understanding of SCS-activated cortical areas/circuits may lead to a more effective and accurate use of SCS for chronic pain relief.

4. Clinical Application and Side Effect Concerns

Although the mechanisms underlying SCS-induced pain relief are still not fully understood, this therapy has been used for management of pain for almost half a century [4].

4.1. SCS Effects in PNP. With a growing number of clinical trials, it is now obvious that its efficiency varies with the clinical indication tested like CRPS, FBSS, diabetic neuropathy, ischaemic pain, and postherpetic neuralgia [72–77].

The most optimal effectivity of SCS-induced pain relief was reported in a randomized trial of CRPS type 1 patients [73]. This study reported 14 (58%) out of 24 patients implanted with SCS devices (delivering conventional stimulation at 85 Hz) together with physical therapy showed a remarkable and significant reduction (24%) of pain score (visual analogue scale (VAS)) as compared to those patients treated with physical therapy only. Another indication of SCS for pain treatment is FBSS [75, 78, 79]. A randomized controlled trial of 50 patients with SCS-device implantation (offering conventional stimulation at 49 Hz) illustrated that 48% of the patients achieved pain relief in legs in the intention-to-treat at 6 months, which was significantly more efficient than that (9%) of the conventional medical management group [75]. To improve the responsive rate of SCS, more stimulation programs have been developed recently. For example, high-frequency stimulation (10 kHz) or burst stimulation reduced VAS in 14 out of 16 refractory FBSS patients, which means the responsive rate can reach to 88% [80]. For FBSS patients whose back pain reduction was less than 50% after trial SCS, additional subcutaneous stimulation to SCS significantly suppressed their back pain compared to those controls with subcutaneous lead implantation but switched off [81]. Moreover, a recent study on the effectiveness of SCS in patients with painful diabetic neuropathy (PDNP) provided evidence that SCS over a six-month period could significantly reduce the pain scores during both daytime and nighttime, compared to most efficient pain relief induced by medical treatment [76]. Another randomized clinical trial showed that the average VAS score of PDNP patients was significantly reduced by six months of treatment of SCS from 73 to 31 ($n = 40$), whereas there was no change in the control group (VAS score = 67; $n = 20$) [82]. Additionally, peripheral ischaemic pain due to diabetes and ischaemic ulceration in 25 patients was significantly attenuated by SCS during an 18-month follow-up period, compared to the control group [72]. Furthermore, an investigation over 29 months (median) found that SCS provided a long-term pain relief in 23 out of 28 patients suffering refractory postherpetic neuralgia for more than 2 years [74]. A recent clinical study reported that 60-70% of patients diagnosed as CRPS, and postherpetic neuralgia had a significant lower VAS score after 12-month SCS treatment, compared to baseline [77]. Moreover, as we have known that not all the patients have responses to SCS, about 2/3 of CRPS patients do not respond to SCS [37], and PDNP patients are susceptible to infection; therefore, a trial period of SCS is required before permanent device implantation.

Taken together, the European Federation of Neurological Societies (EFNS) and the IASP Special Interest Group on Neuropathic Pain (NeuPSIG) gave SCS a weak recommendation for CRPS and FBSS [83, 84]. The European Academy of Neurology also weakly recommended SCS for diabetic painful neuropathy [85]. However, the National Institution of Health and Care Excellence (NICE) published a guide for UK and most of Europe and recommended SCS as an option for treating chronic pain of neuropathic and ischaemic origin [86].

4.2. SCS Effects in CNP. A literature review about the clinical practice of SCS for treatment of SCI-induced neuropathic pain by searching MEDLINE and EMBASE databases showed that 9 out of 22 case studies reported more than 50% pain relief was achieved by conventional stimulation, 3 out of 22 reported 30-80% pain reduction was obtained by high-frequency stimulation, and 1 out of 22 reported 30% pain score was reduced by burst stimulation. Although the quality of these case studies was low, they support the pain-relief efficacy and safety of SCS in SCI and point out the possibility of clinical practice of SCS for the management of NP after SCI [87]. Together with the preclinical study of SCS in the SCI pain model, which reported that a low-frequency (10-25 Hz) stimulation was associated with neural progenitor activation in the spinal cord and led to long-lasting analgesia [62], conventional SCS with lower frequency might be more efficient in SCI patients. Moreover, the timing of SCS during development of NP is also important for increasing the responsive rate. Early SCS (24 h after nerve injury) increased the number of responders and the duration of analgesic effect than late SCS (16 d after injury) [88], suggesting that SCS should be practiced as early as possible (i.e., the sooner, the better). On the other hand, as an invasive treatment, the risk of complications of SCS due to lead infection and dislocation is unneglectable. Evidence showed that 32% of patients developed device-related complications after 12-month implantation [75]. The adverse events in cervical SCS treatment for chronic pain were hardware malfunction (17.8%), lead migration (13.9%), and lead breakage (6.7%) [10]. A rare complication of SCS that can occur using leads placed via open surgical approach is spinal and radicular compression symptoms caused by the growth of fibrotic epidural mass at the level of the lead [11]. Spinal hematoma due to paddle leads for SCS was also found with 0.63% incidence (18/2868 patients) within 30 days following operation [12]. Therefore, the disadvantages of SCS along with the extent of damage in the individual spinal cord-injured patients should be considered carefully before SCS for treatment of CNP.

To date, despite recent progress and insights into mechanisms involved in SCS-induced pain relief in CNP, this therapy is not yet approved for use in clinic and SCI patients. Future studies should be designed to generate robust evidence about the benefits of SCS (including pain relief, function, and quality of life) in the SCI-induced CNP condition.

5. Summary

The analgesic mechanisms of SCS are gradually unveiled with the involvement of the "Gate control" theory, segmental inhibition, the descending inhibitory system, and cortical modulation. However, SCS is still an evidence-based interventional therapy for use in humans. It is recommended for selected indications related to PNP-like patients who experience refractory pain including CRPS and FBSS. Since the majority of mechanism studies on CNP and SCS are based on the experimental contusion/transection-induced SCI pain models, SCS may be considered as a potential therapy to treat trauma-induced CNP in patients, whereas SCI due to degenerative pathologies and somatic infection is a contraindication of SCS.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare no competing financial interest.

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Research Article

Enhanced Temporal Coupling between Thalamus and Dorsolateral Prefrontal Cortex Mediates Chronic Low Back Pain and Depression

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Numerous neuroimaging studies have demonstrated that the brain plasticity is associated with chronic low back pain (cLBP). However, there is a lack of knowledge regarding the underlying mechanisms of thalamic pathways for chronic pain and psychological effects in cLBP caused by lumbar disc herniation (LDH). Combining psychophysics and magnetic resonance imaging (MRI), we investigated the structural and functional brain plasticity in 36 patients with LDH compared with 38 age- and gender-matched healthy controls. We found that (1) LDH patients had increased psychophysical disturbs (i.e., depression and anxiety), and depression (Beck-Depression Inventory, BDI) was found to be an outstanding significant factor to predict chronic pain (short form of the McGill Pain Questionnaire, SF-MPQ); (2) the LDH group showed significantly smaller fractional anisotropy values in the region of posterior corona radiate while gray matter volumes were comparable in both groups; (3) resting state functional connectivity analysis revealed that LDH patients exhibited increased temporal coupling between the thalamus and dorsolateral prefrontal cortex (DLPFC), which further mediate the relationship from chronic pain to depression. Our results emphasized that thalamic pathways underlying prefrontal cortex might play a key role in regulating chronic pain and depression of the pathophysiology of LDH.

1. Introduction

Chronic low back pain (cLBP) is one of the most common reasons for adults to visit the clinic [1, 2]. Clinically, lumbar disc herniation (LDH), which is mainly caused by the degeneration of the lumbar disc annulus or the external pressure force burdened on the disc, is an important cause of cLBP [3]. LDH patients are clinically characterized by extreme pain and emotional comorbidities, such as anxiety and depression, which seriously diminishes the patient's quality of life [4, 5]. Unfortunately, current treatment effect for LDH is unsatisfactory [6]. This is at least partial due to the limited understanding of the underlying mechanisms of chronic pain and psychological effects of LDH.

Neuroimaging studies have concluded that central nervous system is engaged in the development of cLBP, and the brain of chronic pain patients is continuously processing spontaneous pain by integrating information between multiple brain regions, mainly including primary somatosensory cortex (S1), anterior cingulate cortex (ACC), thalamus, insula, and periaqueductal gray (PAG) [6–8]. In particular, numerous studies suggest a critical role of the thalamus in chronic pain processing [9–11]. Structurally, Apkarian et al. found that gray matter density was reduced in the right

thalamus and was closely correlated with the different patterns of pain characteristics for neuropathic and nonneuropathic cLBP, suggesting that the pathophysiology of chronic pain includes the thalamocortical processes [5]. Functionally, Llinás et al. identified that a common mechanism is operant, that is, the abnormal low-frequency oscillations of the thalamocortical network associated with dysregulations and symptoms attributed to various chronic pain conditions [12]. In line with this, studies using functional magnetic resonance imaging (fMRI) have indicated that low-frequency oscillations and abnormal connectivity of the thalamocortical networks are the basis for persistent pain [9, 13].

Notably, among the thalamocortical linkage, some researchers emphasized the functional connectivity of the thalamus with prefrontal cortex (PFC), possibly reflecting individual differences in pain modulation. It is well documented that the mediodorsal thalamus employs a role in the affective dimension of pain, and deficits of the thalamus and PFC coupling have been well documented in some psychiatric conditions, including depression disorder and schizophrenia [14]. Chronic pain and depression are highly intertwined clinically, which could result in longer duration of symptoms and poor prognosis [15]. Given their comorbidity, common mechanisms have been suggested in pain and depression [16]. Many brain regions, such as the thalamus, medial prefrontal cortex, and amygdala, are involved in the central modulation of chronic pain and depression, suggesting a common mechanism for dysregulation of emotional and reward processing [17, 18]. However, until now, the exact neurobiological mechanism of pain and depression remains unknown, which needs to be further clarified. Investigating how thalamic pathways modulate chronic pain and depression in LDH is essential for facilitating the targeted intervention options for patients.

In the current study, combining psychophysics with MRI techniques, we investigated the structural and functional brain alterations in patients with LDH compared with age- and gender-matched healthy controls (HCs). We related these brain alternations with pain intensity as well as pain-related emotional comorbidities in the patient group. We hypothesized that LDH patients (1) exhibited increased psychophysical problems and (2) had structural and functional thalamic abnormalities, which is significantly related to its pain intensity and pain-related emotional comorbidities.

2. Materials and Methods

2.1. Participants. A total of 36 right-handed LDH patients (25 males, mean age 45.11 ± 10.57 years) and 38 age- and gender-matched right-handed HCs (28 males, mean age 43.68 ± 11.86 years) participated in the study. All patients met the following inclusion criteria: (1) diagnostic and radiological evidence (CT or MRI) for LDH was confirmed by 3 experienced clinicians, (2) radiating pain > 3 score (assessed by visual analogue scale (VAS)); and (3) duration of pain > 3 months. All enrolled patients did not take any painkillers at least one week before the MRI scan. None of the participants had a past or current diagnosis of any psychiatric or major neurological illness. Participants signed the informed consent prior to the experiment on the premise of fully understanding the content of the experiment. The local ethics committee at the Third Affiliated Hospital of Henan University of Chinese Medicine approved the study. All participants were recruited from 2018 to 2019. For the demographic data, group differences in age and education level were evaluated using independent-sample t-tests, and differences in gender were assessed using chi-square test.

2.2. Pain Characteristics. Pain characteristics were assessed using the short form of the McGill Pain Questionnaire (SF-MPQ) [19] and pain sensitivity questionnaire (PSQ) [20], and all participants completed the assessments before the MRI scan. The SF-MPQ comprises (1) a pain rating index (PRI), which includes 15 descriptors, and the range is divided into 4 levels from 0 (none) to 3 (severe); (2) a present pain intensity (PPI) index ranging from 0 (no pain) to 5 (unbearable pain), which evaluates the present pain intensity; and (3) a visual analogue scale (VAS) to assess the average daily pain intensity in the past two weeks. The SF-MPQ total score is the sum of these three subscales. PSQ consists of 17 items. It has been widely used to evaluate the participants' pain sensitivity and has been proven to have good reliability and validity [20].

2.3. Psychophysical Characteristics. All participants completed psychological questionnaires to assess depression (Beck-Depression Inventory, BDI) as well as state and trait anxiety (State-Trait Anxiety Inventory, STAI) [21]. In addition, debriefing of participants includes questionnaires on sleep quality (Pittsburgh sleep quality index, PSQI) and pain vigilance (pain vigilance and awareness questionnaire, PVAQ) [22, 23]. The pain and psychophysical characteristics' differences between LDH patients and HCs were performed by using independent-sample t-tests. For LDH patients, Pearson's correlation analyses were adopted to assess the relationship between pain intensity, psychological variables, and thalamus-based functional connectivity.

In order to assess the joint influence psychophysical factors on chronic pain, a multiple linear regression analysis based on a forward stepwise selection procedure was performed. In this analysis, the severity of chronic pain (overall scores of SF-MPQ) was used as the dependent variable, and the sleep disturbances (PSQI) as well as psychological characteristics (SAI, TAI, BDI, and PVAQ) were identified as explanatory variables. First, the forward stepwise selection procedure includes the explanatory variable that could significantly explain the dependent variable ($p < 0.05$). Then, the procedure adjusts by repeatedly adding other explanatory variable (if any) that could significantly improve the fitting of the model ($p < 0.05$), until no one could improve the model. Before performing multiple linear regression analysis, all variables were examined on deviation from normality using Kolmogorov-Smirnov test (SPSS, IBM, Mac version 23.0.0), and no serious deviation from the normality was observed for any variable ($p > 0.05$).

2.4. MRI Acquisition. All MRI images were collected using a 3T Siemens Skyra scanner at the Third Affiliated Hospital of

Henan University of Chinese Medicine. Structural MRI data were acquired using a 3D magnetization-prepared rapid gradient-echo (MPRAGE) T1-weighted sequence (TR/TE = 1900/3.97 ms, FA = 8°, FOV = 192 mm × 192 mm, matrix = 192 × 192, slices = 192, and slice thickness = 1.0 mm). The diffusion data for each subject were obtained using a diffusion-weighted, single shot, spin-echo, EPI sequence (TR/TE = 10200/91 ms, matrix = 96 × 96, FOV = 192 × 192 mm, voxel size = $2.0 \times 2.0 \times 2.0\,mm^3$, 70 axial slices, 2.0 mm slice thickness, b value = 1500, 64 directions, and phase encoding AP). Resting-state fMRI images were collected with an echo planar imaging (EPI) sequence (TR/TE = 2450/30 ms, flip angle: 90°, FOV = 192 mm × 192 mm, matrix = 64 × 64, slice thickness = 3 mm, slices = 44, GRAPPA = 2, and 220 volumes).

2.5. Surface-Based Morphology Analysis (SBM). The structural MRI was analyzed using FreeSurfer (version 5.2.0, http://surfer.nmr.mgh.harvard.edu). The details are described elsewhere [24], and it has been proven to have good accuracy in detecting cortical and subcortical structures [25, 26]. In short, the primary preprocessing includes Talairach transformation, removal of the nonbrain tissue, and segmentation of the grey matter/white matter (GM/WM) tissue. Cortical thickness is the averaged linking distance between the pial and white surfaces along normal vector. Surface area is the total area of triangles that were connected to the vertex. Volume is quantified by cortical thickness and surface area. Moreover, the entire cerebral cortex was parcellated, and a variety of surface-based data, including maps of cortical volume and surface area, were created. Data were resampled onto the FreeSurfer's average surface map according to cortical folding patterns. The cortical map of each participant was smoothed using a 10 cm full-width half-maximum (FWHM) Gaussian spatial smoothing kernel. The subcortical volumes were obtained from the automated procedure for volumetric measures of the brain structures implemented in FreeSurfer [27]. Totally, five subcortical structures (thalamus, caudate, putamen, hippocampus, and amygdala) were extracted and compared between LDH patients and HCs. We performed one-way analyses of covariance (ANCOVA) to detect subcortical volume differences between the two groups after adding the age, gender, and total brain size as covariates [28, 29]. The significant level was set at $p < 0.05$.

2.6. Tract-Based Spatial Statistics (TBSS). Voxel-wise tract-based spatial statistics of diffusion-weight data was analyzed using TBSS, part of the FMRIB Software Library (FSL) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS) [30]. This method identifies a core white matter "skeleton" that is anatomically equivalent across participants. Diffusion data preprocessing include corrections for head movement and eddy currents and brain extraction [31]. Fractional anisotropy (FA) images were created by fitting a tensor model using DTI fit, and then, FA data from all participants were aligned into a common standard space using the nonlinear registration tool FNIRT. Next, the mean FA image was created and thinned to create a mean FA skeleton representing the centers of all tracts common to the group. As Smith et al. suggested, a threshold of FA > 0.2 was applied to the skeleton with the aim of removing voxels of low FA including peripheral small tracts, where there may be high intersubject variability and gray matter [30]. We also controlled the variable of age, gender, and mean FA value by adding them as covariates. A nonparametric permutation testing with 5000 permutations was applied in the TBSS analysis using *Randomise* function of FSL. Threshold-free cluster enhancement (TFCE) with $p < 0.05$ as significant was used to obtain cluster-based statistics corrected for multiple comparisons, a method to enhance the cluster-like structures in the voxel-based data [32, 33]. Significant regions after TFCE correction $p < 0.05$ were thickened for visualization using the TBSS fill script in FSL. The Johns Hopkins University ICBM-DTI-81 white matter labels atlas was used to locate anatomical structures in the MNI152 space.

2.7. Resting-State fMRI Data Preprocessing. Resting-state fMRI image processing and data analyses were performed using FMRI Expert Analysis Tool (FEAT), version 5.98, which is part of the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl/). Preprocessing of resting-state fMRI data includes motion correction using MCFLIRT [34], distortion correction with field map (FUGUE), removal of nonbrain structures using Brain Extraction Tool [31], spatial smoothing using a Gaussian kernel with a 5 mm FWHM, and high-pass temporal filtering (cutoff: 100 s). Time-series autocorrelation was performed using FMRIB's Improved Linear Model (FILM). Each participants' functional MRI image was coregistered to structural image using a boundary-based registration tool [35]. Then, structural image was normalized to a standard template (MNI152-2 mm) using a linear registration (FLIRT) [34], followed by a nonlinear registration (FNIRT).

The fMRI data underwent a 2-step quality checking method by researchers. First, data were excluded if it were of poor quality due to movement (>1 mm). Second is the warp distortion amount for BBR-based function-to-structure realignment as measured by the minimal cost of the head motion as measured by the root mean square of frame-wise displacement. Three participants (two for LDH patients and one for HCs) were excluded because of head movement during the fMRI scan. In addition, four patients were excluded because of illness during scanning.

2.8. Seed-Based Functional Connectivity Analysis. The thalamus was chosen as a seed for the resting-state functional connectivity analyses, and the seed was identified based on the Harvard Oxford subcortical structural atlas (90% threshold), which are population-based probability atlas in MNI-152 standard space [36]. The mask was first transformed into individual functional space via inverted registration files using applywarp. Average BOLD time courses from the seed region for each individual were extracted using standard methods.

Voxel-wise seed-based functional connectivity analysis was completed using standard methods with a general linear model (GLM) framework [37]. Nuisance regions of interest for CSF and WM were generated for each subject. The mean time series of each individual's seed was set as a connectivity EV with realignment parameters, and the signals of CSF and

Table 1: Demographic and pain characteristics between LDH patients and HCs (mean ± SD).

	LDH patients (36) Mean ± SD	HCs (38) Mean ± SD	χ^2/t value	p value
Female/male	11/25	10/28	0.163	0.686
Age, year	45.11 ± 10.57	43.68 ± 11.86	0.545	0.588
Education, year	11.44 ± 4.18	13.00 ± 3.13	1.818	0.073
PRI	10.67 ± 4.93	0.07 ± 0.27	13.228	<0.001
PPI	2.97 ± 0.97	0.15 ± 0.37	16.651	<0.001
VAS	5.72 ± 1.56	0.26 ± 0.64	19.858	<0.001
SF-MPQ	19.36 ± 6.41	0.50 ± 1.20	17.828	<0.001
PSQ	66.69 ± 23.25	53.79 ± 21.30	2.485	0.015
BDI	10.03 ± 6.58	2.92 ± 3.44	5.866	<0.001
SAI	31.28 ± 6.57	26.63 ± 5.49	3.395	0.001
TAI	38.83 ± 8.12	32.16 ± 6.11	3.985	<0.001
PSQI	11.86 ± 7.71	6.16 ± 4.59	3.893	<0.001
PVAQ	42.24 ± 12.63	34.03 ± 15.13	2.461	0.016

PRI: a pain rating index; PPI: a present pain intensity; VAS: 10 cm visual analogue scale; SF-MPQ: short form of the McGill Pain Questionnaire; PSQ: pain sensitivity questionnaire; BDI: Beck-Depression Inventory; SAI: state-anxiety index; TAI: trait-anxiety index; PSQI: Pittsburgh sleep quality index; PVAQ: pain vigilance and awareness questionnaire; LDH: lumbar disc herniation; HCs: healthy controls.

WM were set as nuisance regressors. The parameter estimates and variances served as input to group analysis in FSL's feat using a mixed-effect FLAME approach (FLAME, FMRIB's Local Analysis of Mixed effects). The group-level statistical images were corrected using parametric family-wise error (FWE) method at cluster level, determined by $Z > 2.3$, and a corrected cluster significance threshold of $p < 0.05$. A two-group unpaired t-test was used for comparing differences in the thalamus-based functional connectivity between LDH patients and HCs. The group-level statistical images were corrected using parametric family-wise error (FWE) method at cluster level, determined by $Z > 2.3$, and a corrected cluster significance threshold of $p < 0.05$.

Additionally, thalamus has complex substructures, and we also performed seed-based functional connectivity analyses for different subregions of thalamus. We adopt the probability atlas of 7 subthalamic regions provided by FMRIB, which were segmented according to their white matter connectivity to cortical regions. Two different subregions of thalamus were selected as seeds for the resting-state functional connectivity analyses, which mainly connect the prefrontal and the somatosensory regions, respectively.

2.9. Mediation Analysis. The functional connectivity between thalamus and DLPFC was identified to be significantly related to SF-MPQ as well as BDI ratings. Therefore, to further investigate the relationship between chronic pain intensity, depression, and thalamus-DLPFC functional connectivity, a bootstrapped mediation analysis was finally employed. The mediation analyses were performed using the SPSS (IBM, version 23.0.0) version of the PROCESS macro (http://www.processmarcro.org, version 2.16.3). We tested two models: (1) independent variable was SF-MPQ, BDI was the dependent variable, and functional connectivity of thalamus-DLPFC was the mediator; (2) independent variable was BDI, SF-MPQ was the dependent variable, and functional connectivity of thalamus-DLPFC was the mediator. These analyses determined the indirect effects of thalamus-DLPFC functional connectivity on chronic pain and depression, yielding the 95% confidence intervals (CIs) of the indirect effects. A significant mediation occurs when the 95% confidence intervals did not include zero [38].

3. Results

3.1. Psychophysics. The comparison of demographic, pain, and psychological characteristics between LDH patients and HCs is presented in Table 1. The age, gender, and education levels were well matched between the two groups. As expected, the intensity and sensitivity of pain, as quantified by PRI ($t = 13.228$, $p < 0.001$), PPI ($t = 16.651$, $p < 0.001$), VAS ($t = 19.858$, $p < 0.001$), total SF-MPQ scores ($t = 17.828$, $p < 0.001$), and PSQ score ($t = 2.485$, $p = 0.015$) were significantly larger in LDH patients than in HCs (Table 1 and Figure 1(a)). Furthermore, patients reported higher levels of depression ($t = 5.866$, $p < 0.001$) as well as state and trait anxiety ($t = 3.395$, $p = 0.001$; $t = 3.985$, $p < 0.001$; Table 1 and Figure 1(b)). In addition, sleep quality (PSQI, $t = 3.893$, $p < 0.001$) and pain vigilance (PVAQ, $t = 2.461$, $p = 0.016$) were significantly higher in LDH patients than in HCs (Table 1 and Figure 1(b)). Characteristics of patients with LDH are shown in Supplementary Table S1.

3.2. Relationship between Pain Intensities and Psychophysical Variables. For LDH patients, BDI ratings were significantly positively related with SF-MPQ ratings ($r = 0.675$, $p < 0.001$, Figure 2(a)), while both SAI and TAI ratings were not significantly related with SF-MPQ ratings (SAI vs. SF-MPQ: $r = 0.274$, $p = 0.106$ and TAI vs. SF-MPQ: $r = 0.240$, $p = 0.165$, Figure 2(a)). In addition, PSQ, PSQI, and PVAQ

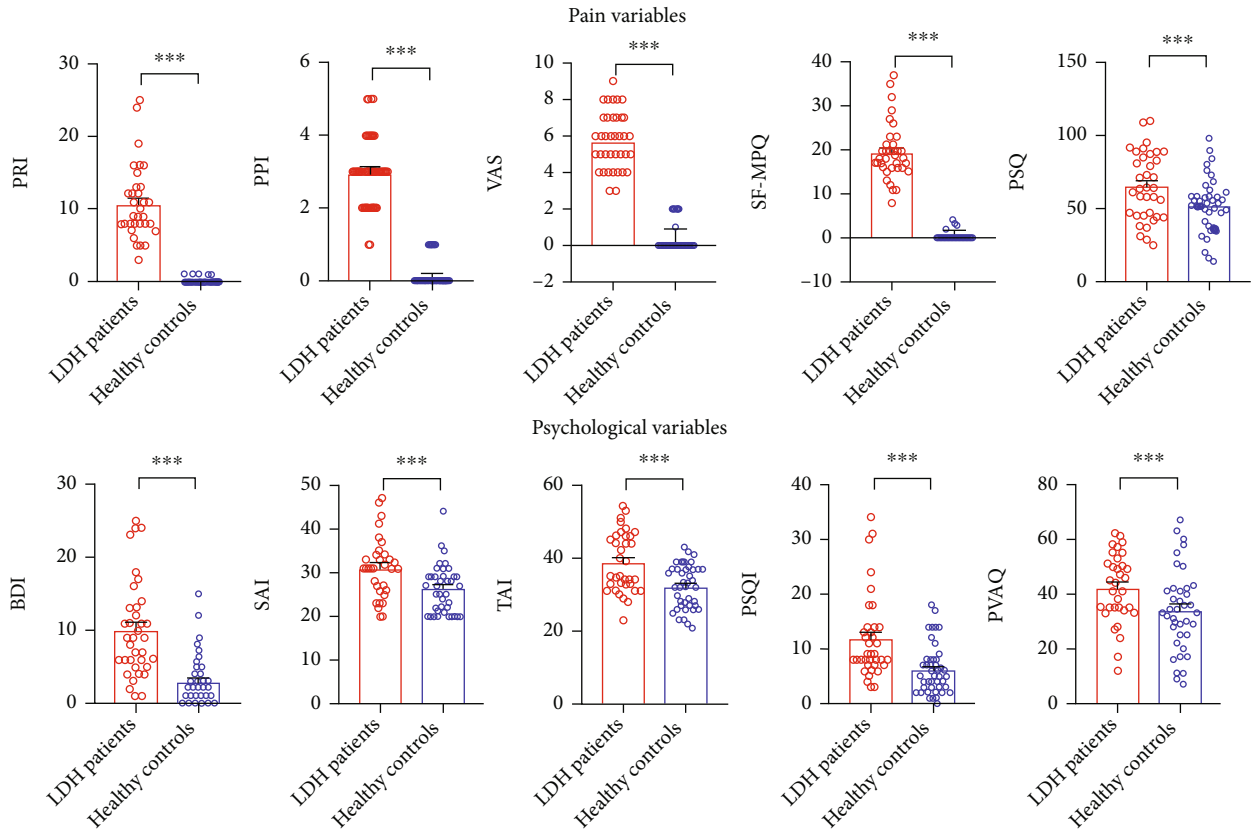

Figure 1: Comparison of pain intensities (i.e., PRI, PPI, VAS, and SF-MPQ), pain sensitivity (PSQ), and psychological variables (i.e., BDI, SAI, TAI, PSQI, and PVAQ) between LDH patients and HCs. (a) Pain intensity (i.e., PRI, PPI, VAS, and SF-MPQ) and pain sensitivity ratings (PSQ) were significantly larger in LDH patients than in HCs. PRI: a pain rating index; PPI: a present pain intensity; VAS: 10 cm visual analogue scale; SF-MPQ: short form of the McGill Pain Questionnaire; PSQ: pain sensitivity questionnaire. (b) Psychophysical variables (i.e., BDI, SAI, TAI, PSQI, and PVAQ) were significantly larger in LDH patients than in HCs. BDI: Beck-Depression Inventory; SAI: state-anxiety index; TAI: trait-anxiety index; PSQI: Pittsburgh sleep quality index; PVAQ: pain vigilance and awareness questionnaire; LDH: lumbar disc herniation.

ratings were significantly positively correlated with SF-MPQ ratings in LDH patients (PSQ vs. SF-MPQ: $r = 0.471$, $p = 0.003$; PSQI vs. SF-MPQ: $r = 0.434$, $p = 0.008$; and PVAQ vs. SF-MPQ: $r = 0.407$, $p = 0.018$, Figure 2(b)).

3.3. Multiple Linear Regression Model of Clinical Pain Predictors in LDH Patients. For LDH patients, multiple linear regression analysis revealed that the dependent variable SF-MPQ was significantly influenced by the explanatory variable of BDI (accounting for 47.90% of the variability; standardized $\beta = 0.704$, $t = 5.520$, and $p < 0.001$), whereas not significantly affected by the explanatory variable of SAI (standardized $\beta = 0.101$, $t = 0.766$, and $p = 0.450$), TAI (standardized $\beta = -0.215$, $t = 1.473$, and $p = 0.151$), PSQI (standardized $\beta = 0.234$, $t = 1.613$, and $p = 0.117$), and PVAQ (standardized $\beta = 0.152$, $t = 1.099$, and $p = 0.280$).

3.4. Gray Matter Volume and Tract-Based Spatial Statistics. No significant differences in cortical and subcortical volumes were detected between LDH patients and HCs after adding the age, gender, and total brain size as the controlled variables. Subcortical volume comparisons between the two groups are presented in Supplementary Table S2.

Compared with HCs, FA values in the region of posterior corona radiate (PCR) were significantly smaller in LDH patients (Figure 3). As chronic pain was obviously predicted by depression in LDH patients, we further conducted correlation analyses among pain intensity, depression, and FA abnormalities in the patient group. No significant correlations were observed between FA values and SF-MPQ ($r = -0.274$, $p = 0.106$) as well as BDI ratings ($r = -0.236$, $p = 0.165$) in LDH patients.

3.5. Seed-Based Functional Connectivity. When the thalamus was used as the seed, resting-state functional connectivity demonstrated that thalamus exhibited stronger functional connectivity with the DLPFC, anterior cingulate cortex (ACC), insula, and posterior cingulate cortex (PCC) in LDH patients than in HCs ($Z > 2.3$, $p < 0.05$ cluster-wise corrected, Figure 4(a)). Further, we extracted abnormal thalamus-based functional connectivity and conducted correlation analyses to assess their relationship with SF-MPQ and BDI ratings in LDH patients. Results indicated that thalamus and DLPFC coupling was negatively correlated with both SF-MPQ and BDI ratings (SF-MPQ: $r = -0.374$, $p = 0.029$ and BDI: $r = -0.434$, $p = 0.010$, Figure 4(b)); thalamus and PCC

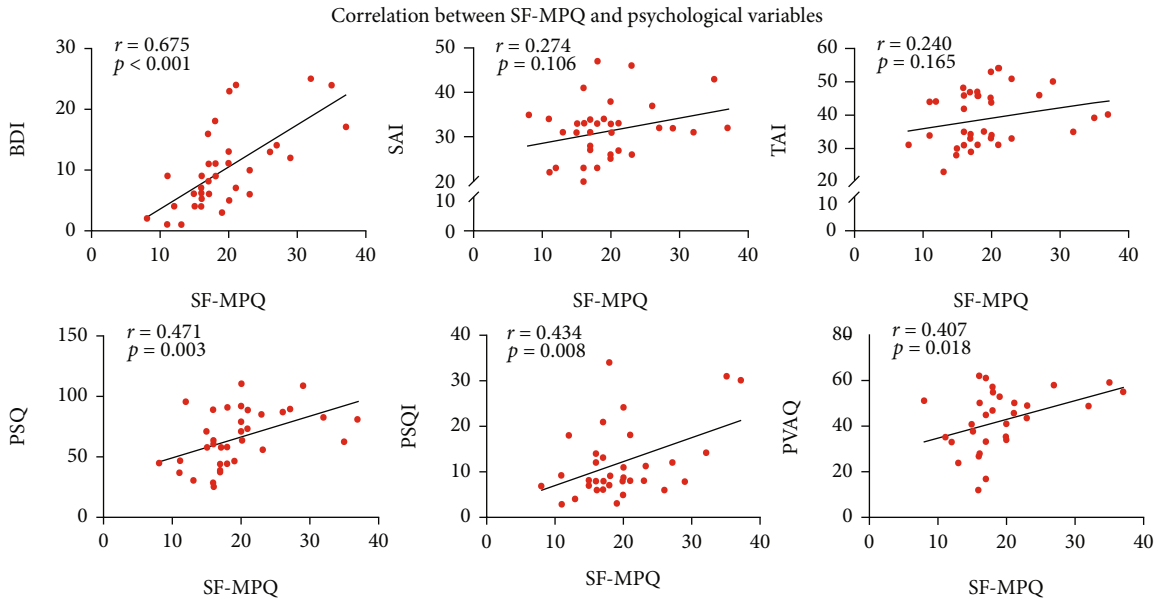

Figure 2: Correlation between pain intensities and psychological variables in LDH patients. (a) SF-MPQ ratings were significantly correlated with BDI, but not with SAI and TAI in LDH patients. SF-MPQ: short form of the McGill Pain Questionnaire; BDI: Beck-Depression Inventory; SAI: state-anxiety index; TAI: trait-anxiety index. (b) SF-MPQ ratings were significantly correlated with PSQ, PSQI, and PVAQ in LDH patients. PSQ: pain sensitivity questionnaire; PSQI: Pittsburgh sleep quality index; PVAQ: pain vigilance and awareness questionnaire.

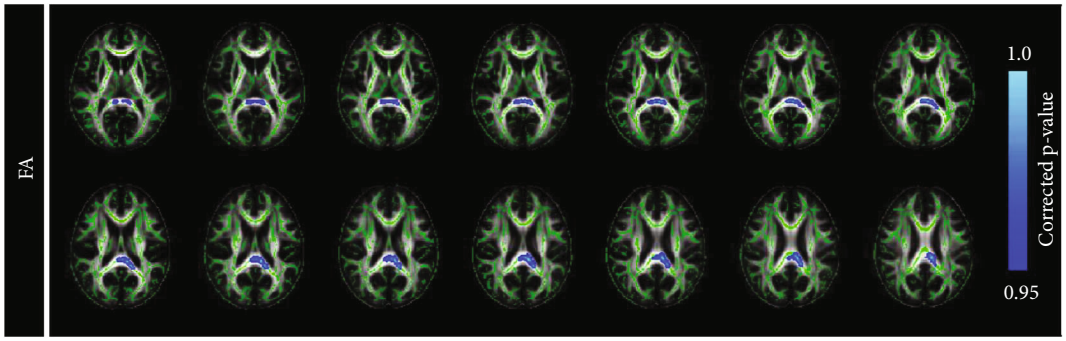

Figure 3: Tract-based spatial statistics maps are shown the FA differences between the two groups, and blue represents regions with significantly decreased FA in LDH patients, compared with HCs.

coupling was negatively correlated only with BDI ratings ($r = -0.365$, $p = 0.034$), but not with SF-MPQ ratings ($r = -0.217$, $p = 0.177$, Figure 4(b)). Additionally, functional connectivity between thalamus and insula and between thalamus and ACC was negatively correlated with BDI ratings, but with a marginal significance (thalamus-insula vs. BDI: $r = -0.329$, $p = 0.058$ and thalamus-ACC vs. BDI: $r = -0.293$, $p = 0.093$), whereas functional connectivity between thalamus and insula and between thalamus and ACC was not correlated with SF-MPQ ratings (thalamus-insula vs. SF-MPQ: $r = -0.169$, $p = 0.338$ and thalamus-ACC vs. SF-MPQ: $r = -0.248$, $p = 0.171$). In addition, the results of the resting-state functional connectivity of subregions for the thalamus are presented in Supplementary Fig. S1.

3.6. Mediation Analysis. Given that thalamus-DLPFC functional connectivity is correlated with both pain and depression ratings, we next tested the indirect effects of thalamus-DLPFC functional connectivity on chronic pain and depression. The thalamus-DLPFC temporal coupling mediated the relationship from SF-MPQ to BDI (direct effect = 0.617, $p < 0.001$; indirect effect = 0.092, 95% confidence interval: [0.002, 0.306], Figure 5(a)). In contrast, the thalamus-DLPFC temporal coupling did not mediate the relationship from BDI to SF-MPQ (direct effect = 0.038, $p > 0.05$; indirect effect = −0.095, 95% confidence interval: [-0.259, 0.113], Figure 5(b)). These results suggest that the thalamus-DLPFC coupling plays an important role in the modulation of chronic pain, possibly affecting individuals' perception of pain through regulating their depression levels.

4. Discussion

LDH is a chronic pain syndrome that is mainly caused by the degeneration of the lumbar disc annulus or the external pressure force burdened on the disc. The mechanism of the

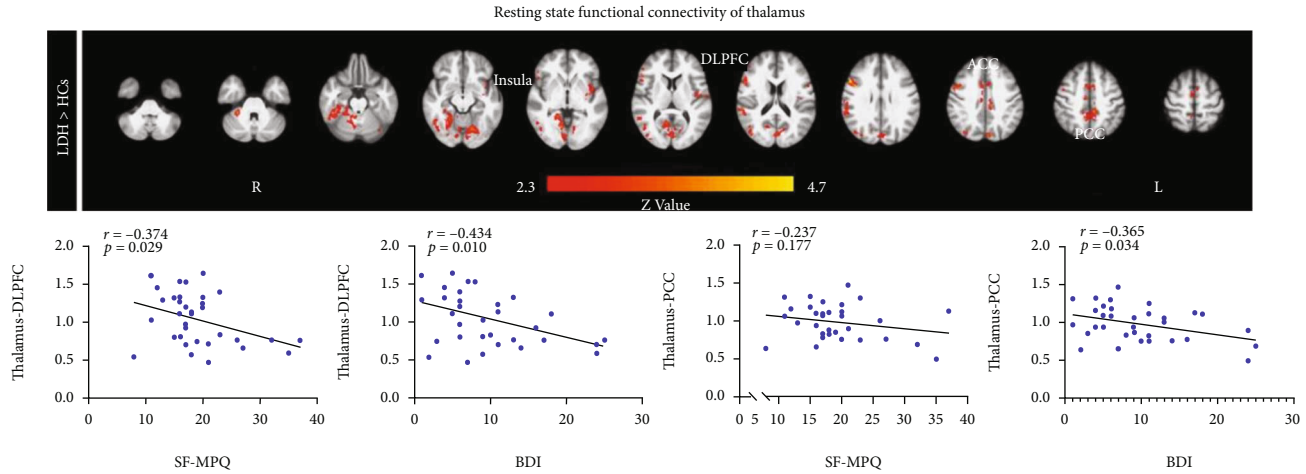

Figure 4: Resting-state functional connectivity of the thalamus and correlations between thalamus-based functional connectivity and SF-MPQ as well as BDI ratings. (a) Thalamus showed increased resting-state functional connectivity with the DLPFC, ACC, PCC, and insula in LDH patients than in HCs. (b) For LDH patients, resting-state functional connectivity between thalamus and DLPFC was negatively correlated with SF-MPQ and BDI ratings, and resting-state functional connectivity between thalamus and PCC was negatively correlated with BDI, but not with SF-MPQ ratings. DLPFC: dorsolateral prefrontal cortex; ACC: anterior cingulate cortex; PCC: posterior cingulate cortex; SF-MPQ: short form of the McGill Pain Questionnaire; BDI: Beck-Depression Inventory.

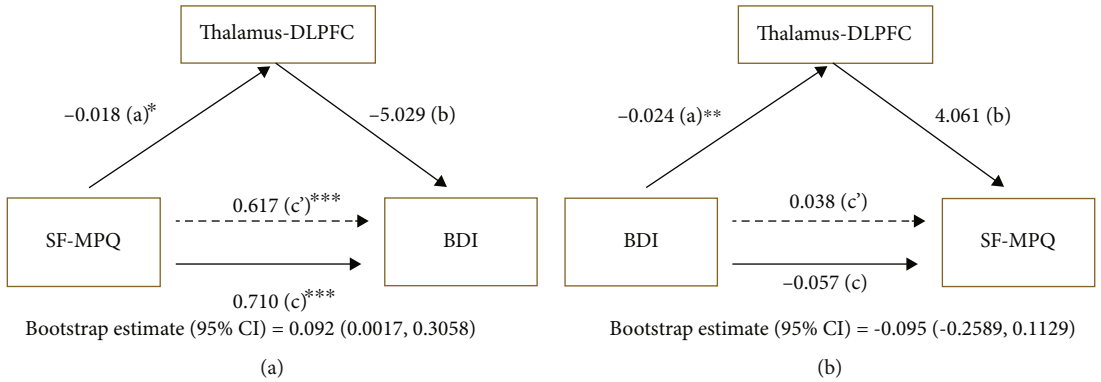

Figure 5: Mediation analysis. (a) The thalamus-DLPFC temporal coupling mediated the relationship from SF-MPQ to BDI. Path c is the total effect of SF-MPQ on BDI; path c' is the direct effect of SF-MPQ on BDI after controlling for the thalamus-DLPFC functional connectivity; the product of a and b (ab) is the indirect effect of SF-MPQ through the thalamus-DLPFC functional connectivity on BDI. (b) In contrast, the thalamus-DLPFC temporal coupling did not mediate the relationship from BDI to SF-MPQ. Path c is the total effect of BDI on SF-MPQ; path c' is the direct effect of BDI on SF-MPQ after controlling for the thalamus-DLPFC functional connectivity; the product of a and b (ab) is the indirect effect of BDI through the thalamus-DLPFC functional connectivity on SF-MPQ. SF-MPQ: short form of the McGill Pain Questionnaire; BDI: Beck-Depression Inventory. DLPFC: dorsolateral prefrontal cortex. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

underlying thalamic pathway regulation of chronic pain and psychological effects in cLBP caused by lumbar disc herniation (LDH) is poorly addressed. In the present study, we investigated the relationship between brain structural/functional plasticity and the degree of chronic pain as well as pain-related psychological factors in LDH patients, and our findings can be summarized as follows: (1) LDH patients exhibited severe psychophysical disturbs (i.e., depression and anxiety), and depression was found to be an outstanding significant factor to predict chronic pain; (2) we did not observe significant structural plasticity changes for LDH patients, in terms of cortical thickness and subcortical volumes. FA values in LDH patients were identified to be significantly decreased only in the region of posterior corona radiate (PCR) but not correlated with chronic pain or any psychological factors; (3) the main finding of this study is that the functional connectivity between the thalamus and DLPFC was significantly correlated with the subjective ratings of SF-MPQ and BDI in LDH patients, and critically, the thalamus-DLPFC coupling mediated the relationship from chronic pain to depression. Our results suggested that thalamic pathways underlying prefrontal cortex might play a key role in regulation chronic pain and depression of the pathophysiology of LDH and may lead to optimized treatments in clinical practice.

4.1. Association between Chronic Pain and Psychophysical Characteristics in LDH. In addition to the primary

etiological cause, LDH, as a typical chronic low back pain, has profound and prolonged psychophysiological consequences, such as increased depression, as demonstrated by the analysis of the multiple linear regression. Of note, different types of chronic pain conditions have been well described in a large of literature to be comorbid with psychological disorders [39]. Indeed, chronic pain and depression are commonly coexisting, and inevitably common mechanisms have been proposed. In the current study, we found that depression is the outstanding significant factor to predict chronic pain, while other psychophysical characteristics (i.e., SAI, TAI, PSQI, and PVAQ) are not significant. Depression, which was considered to be comorbidity of chronic pain, could aggravate the severity of pain during its chronicity process [14]. In clinical applications, the difficulty of relieving pain without eliminating its comorbidities has become a broad consensus. Our results emphasized the complexity of LDH, which would pose new challenges for the comprehensive assessment and accurate diagnosis of this chronic pain.

4.2. Structural Plasticity and Its Relationship with Psychophysical Characteristics in LDH. Structural morphology analyses demonstrated that cortical and subcortical gray matter volumes were comparable between the two groups. In addition, we assessed microstructure properties by using the voxel-wise tract-based spatial statistics method. FA is the most frequently used parameter in DTI studies, and it reflects structural integrity and geometry of axonal fibers [40]. And a different pattern in LDH patients compared with HCs was present that decreased FA values was observed in the region of PCC in LDH. Lower FA was proposed to be correlated with local cerebral edema, cerebrospinal fluid, compromised myelin structure, changes in axonal morphologic structure, and altered interaxonal spacing of fiber bundles [41]. Reduced FA values in the PCC suggest some degree of demyelination, inflammation, edema, or changes in axon count, density, diameter, or degree of crossing [42]. Since PCC is associated with pain perception, our data suggest that several aspects of pain processing and regulation may be affected in LDH. Unfortunately, we did not find significant correlations between FA values and SF-MPQ as well as BDI ratings in LDH patients. These results suggest that the chronic pain and negative psychological aspect development of LDH might not have major influence on the structural plasticity of the brain.

4.3. Alterations of Thalamus Connectivity and Its Relationship with Psychophysical Characteristics in LDH. Compared to HCs, LDH patients exhibited a significantly greater resting-state functional connectivity between thalamus with several brain regions, including DLPFC, ACC, insula, and PCC. Thalamus is a main gateway of nociceptive inputs to the cerebral cortex, and deficits of this region may be a reason for generalized sensory abnormalities commonly related to chronic pain [43]. Henderson et al. identified altered functional connectivity between the thalamus and cortical regions including S1, S2, and anterior insula in chronic pain patients, suggesting that chronic pain is associated with altered thalamic activity [9, 11]. The frontal lobe is an important structure involved in pain, and this structure involves the modulation of pathological algesthesia in the formation input and central sensitization [44]. Moreover, we demonstrated that the strength of temporal coupling between the thalamus and DLPFC mediated the relationship from chronic pain to depression. In consistent with previous publications, in which thalamus showed stronger resting-state function connectivity with frontal cortex when the intensity of chronic pain increased, our observations also suggested that the deficits of the ascending pain modulation system were highly associated with the intensity of chronic pain and its emotional comorbidity of depression [45].

4.4. Association between Chronic Pain and Depression Was Mediated by the Thalamus-DLPFC Functional Connectivity in LDH. Previous studies of chronic pain have reported consistent findings with respect to the assocaitions between clinical pain and negative emotional symptoms as well as thalamus-related functional connectivity [5, 11]. Clinical pain and negative emotional symptoms are typically defined in terms of clinical assessments. Especially, mediation analyses revealed the modulation of SF-MPQ on BDI was mediated by the thalamus-DLPFC coupling, while the modulation of BDI on SF-MPQ was not. The thalamus is the main gateway to the cerebral cortex, relaying information to specific cortical regions. Approximately 25% of the spinothalamic tract fibers terminate in the medial thalamus and then project mainly to the cingulate cortex and prefrontal cortex [46]. Several lines of evidence suggest a role for the DLPFC in the suppression of pain and maintenance of pain inhibition [47, 48]. Brascher et al. revealed that uncontrollable pain lead to increased activation of pain-related regions including the thalamus, but that DLPFC had increased negative connectivity strength during controllable pain to the thalamus, suggesting the DLPFC suppressed thalamus activity and reduced pain sensitization associated with uncontrollable pain [49]. In line with previous reports, our observations suggested that enhanced thalamus-DLPFC coupling might generate a pain suppression, thereby reducing the emotional comorbidity of depression. In addition, the DLPFC is also involved in cognitive components of the pain experience while the mediodorsal thalamus plays a role in the affective dimension of pain [48, 50, 51], and the link between chronic pain and the DLPFC-thalamus coupling could reflect persistent attempts to regulate pain. It is well documented that pain is a major risk factor for depression, and depression can exacerbate chronic pain progression [39]. Our data suggests that clinical pain affected depression indirectly through thalamus-DLPFC functional connectivity, which implies that the degree to which chronic pain states alter normal function of these circuits depends on the severity of pain in a given patient. Otherwise, the modulation of depression on pain could be attributed to different neural systems.

4.5. Limitations. First, the sample size of patients is limited and involves patients with varying severity and duration of disease, which limited the investigation of the detailed role of each mechanism in the development of LDH. Second,

only longitudinal studies in larger samples allow us to track the development of the key variables involved over time to shed light on their relationship and develop a causal model between pathopsychophysiological factors and chronic pain severity in LDH. Third, the sample size involving LDH patients is relatively small, which limits our ability to explore in different thalamic nuclei, so that it cannot provide a precise description of the thalamocortical abnormality in LDH patients. Future research using large sample data from ultrahigh-field imaging to dissect the functional neuroanatomy of the thalamus into its components will help determine possible differences in thalamic function related to chronic pain between LDH patients and HCs. Finally, it is not clear whether LDH patients were characterized with possible transmission mechanism from the periphery to the spinal cord, which needs to be elucidated by future work with combined spinal-brain fMRI.

4.6. Further Prospects. Despite the limitations, this study provides further evidence that the modulation of chronic pain on depression was mediated by enhanced functional connectivity between thalamus and DLPFC. Future studies still need large sample sizes or longitudinal studies to replicate and generalize these results across large chronic pain patients. More detailed insights into the structural and functional brain plasticity underlying LDH will shed light on the development of new more targeted treatment options in clinical practice.

Data Availability

The data and codes that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

Conflicts of Interest

The authors have no conflict of interest to declare.

Authors' Contributions

Hong Li was responsible for data collection, data analysis, and writing the manuscript. Qiaoyan Song and Ruya Zhang were responsible for data collection. Youlong Zhou was the advisor for study protocol. Yazhuo Kong was responsible for the protocol design, study monitoring, editing, and approval of the manuscript.

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Supplementary Materials

Table S1: characteristics of LDH patients. Table S2: comparison of subcortical volumes between LDH patients and HCs (mean ± SD). Fig.S1: resting-state functional connectivity of the subregions for thalamus. *(Supplementary Materials)*

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Research Article

Impaired Cognitive Empathy in Outpatients with Chronic Musculoskeletal Pain: A Cross-Sectional Study

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Background. In recent years, a growing number of researchers showed significant interest in psychological and social interventions to manage chronic musculoskeletal (MSK) pain. Cognitive and emotional empathy is an attractive and valuable sociopsychological factor that may provide protection and resilience against chronic MSK pain. However, its effect on outpatients remains underexplored. *Objective*. To compare the empathy ability between chronic MSK pain outpatients and healthy controls and explore the relationship between cognitive/emotional empathy and chronic pain. *Methods*. Patients with chronic MSK pain ($n = 22$) and healthy controls ($n = 26$) completed the pain assessment and empathy ability task, utilizing a multidimensional empathy assessment tool with satisfactory reliability and validity (i.e., the Chinese version of the Multifaceted Empathy Test (MET-C)). *Results*. The data indicated that the chronic MSK pain outpatients had impaired cognitive empathy (i.e., lower squared cognitive empathy accuracy: Student's $t = -2.119$, $P = 0.040$, and longer task completion time: Student's $t = 3.382$, $P = 0.002$) compared to healthy controls, and cognitive empathy was negatively correlated with pain intensity ($r = -0.614$, $P = 0.002$). Further, the impaired cognitive empathy was present in identifying positive, but not negative emotions. *Conclusion*. These results indicate that chronic MSK pain is associated with impaired empathy ability. Our studies contribute to offering a potential direction for developing psychosocial interventions to treat chronic MSK pain.

1. Introduction

Chronic musculoskeletal (MSK) pain is the main contributor to disability worldwide [1]. According to the World Health Organization (WHO), 20–33% of the world's population (1.75 billion people) has some form of chronic MSK pain [1]. Chronic MSK pain was commonly defined as pain persisting for longer than 3 months, and it may be due to sustained stimulation of nociceptors damaged in areas of persistent tissue damage (i.e., bones, muscles, ligaments, tendons, and even nerves) [2]. Chronic MSK pain results in great suffering among patients and poses an immense global socioeconomic burden [3]. Although it increases suffering in daily activities, drug consumption, and high frequency of sick

leave and disability pensions, there is no consensus on the mechanism underlying chronic MSK pain, and the current targeted medical treatments have limited efficacy; therefore, further research on chronic MSK pain is required [4].

Most MSK patients have to live with pain for a long duration, and the continuous physical suffering and social stigmatization from MSK pain decrease their quality of life [5]. Chronic MSK pain is also often accompanied by mental health problems, such as depression, anxiety, emotional regulation problems, and sleep disorders, as well as impaired cognitive function (e.g., decreased inhibitory control, memory and, in particular, emotion-related ability), which might impair social function [6, 7]. This social dysfunction and the accumulating chronic pain itself would alter neural circuits involved in cognitive and emotional control, exacerbating the chronic pain or causing a transition to severe neuropathic pain [8, 9]. In this context, the biopsychosocial model, which posits that chronic pain is a multidimensional disorder that involves the interaction of physiological, psychological, and social factors, is the most widely acceptable and reliable theory for chronic pain [10, 11]. In this framework, chronic MSK pain is deemed to be associated with psychological and social processes which, in turn, greatly impact the feeling of pain in muscles and the skeleton [12].

One of the most valuable and attractive indicators of social function is empathy, which is pivotal to social relationships and is an important factor that influences the quality of life [13]. Thus, besides the use of pharmacological interventions (either as monotherapies or combination therapies), empathy ability, which may be a protective factor related to the psychological and social aspects of chronic pain, needs to be assessed and analyzed [14]. Empathy comprises cognitive empathy (mental perspective-taking: emotion recognition and theory of mind) and emotional empathy (vicarious sharing of emotion: affective sharing) [15]. Empathy ability predicted self-perceived social support and positive life changes, which allows resilience in response to chronic pain [16]. Additionally, a study reported that patients with chronic low back pain had impaired empathy (as measured by the Basic Empathy Scale in Adults) [17], and improving empathy ability improves interpersonal relationships and quality of life [18].

However, little empirical evidence demonstrates links between empathy ability and specific dimensions of chronic pain (such as pain intensity and duration) [19], especially for outpatients. In addition, these studies usually assessed empathy using single-dimension questionnaires, so they failed to accurately determine the level of empathy among chronic MSK pain patients [20]. Accordingly, the Multifaceted Empathy Test (MET), which provides a more stable estimation of empathy and involves photorealistic stimuli, is recommended [21]. The use of the MET may deepen clinicians' understanding of patients' cognitive empathy and emotional empathy, contributing to both research and clinical decision making.

We recruited outpatients with chronic MSK pain, which is a major type of chronic pain, in this study. The principal objective was to investigate the multidimensional empathy ability (assessed using the self-reported Interpersonal Reactivity Index (IRI) and the MET-C) of these chronic MSK pain outpatients compared to healthy controls (HCs) and the relationships between chronic MSK pain and pain-related factors (pain duration, pain intensity, sleep quality, and emotion alterations).

2. Materials and Methods

2.1. Participants. Twenty-two outpatients with chronic MSK pain (5 males and 17 females; mean age ± SD, 44.41 ± 7.94 years) and twenty-six healthy people (HCs; 8 males and 18 females, mean age ± SD, 40.08 ± 10.86 years) with the same gender distribution and age range participated in the study. In a brief patient consultation, all detailed information related to each patient's pain was recorded. The criteria for inclusion were (i) with primary chronic MSK pain according to the International Association for the Study of Pain Classification of Chronic Pain for the International Classification of Diseases [22], including the pain in the shoulder, leg, arm, and back; (ii) aged 18-60; (iii) course of disease ≥ 3 months with pain intensity > 3/10 Numerical Rating Scale (NRS); and (iv) provision of informed consent. The exclusion criteria were as follows: (i) other major physical or mental disorders or other types of chronic pain (including neuralgia or visceral pain); (ii) alcohol or drug addiction; (iii) participated in other physical therapy within the past 3 months; (iv) recently received major surgical treatment; and (v) enrolled in any other rehabilitation program. The flow chart of patients is shown in Figure 1.

All participants voluntarily signed informed consent forms prior to recruitment and were compensated $15 after completing all the questionnaires. The study was approved by the Ethics Committee of the Affiliated Jiangsu Shengze Hospital of Nanjing Medical University (JSSZYY-LLSC-202019) and was registered with the China Clinical Trial Registration Center (http://www.chictr.org.cn) under the number ChiCTR2000041062.

2.2. Questionnaires. The participants filled out standardized questionnaires. The questionnaires quantitatively assessed pain and empathy (as described below). To eliminate the effect of interference factors which are common and specific in chronic pain patients, the emotions, sleep quality, and mental state of subjects in both groups were also evaluated using the Positive and Negative Affectivity Scale (PANAS), Pittsburgh Sleep Quality Index (PSQI), and Depression, Anxiety, and Stress Scales–21 Items (DASS-21), respectively [23, 24]. The anatomical pain sites of the patients are presented in Table 1.

2.3. Pain Assessment. We assessed both pain duration and pain intensity, which was evaluated using two pain scales: the 11-point NRS and the Short-Form McGill Pain Questionnaire (SF-MPQ). The NRS is used worldwide as a valid measure of pain intensity with promising clinical value for chronic pain patients [25]; the SF-MPQ allows comprehensive assessment of pain quality (based on sensory and affective dimensions of pain experience) and intensity [26]. The SF-MPQ comprises a list of pain adjectives and is considered

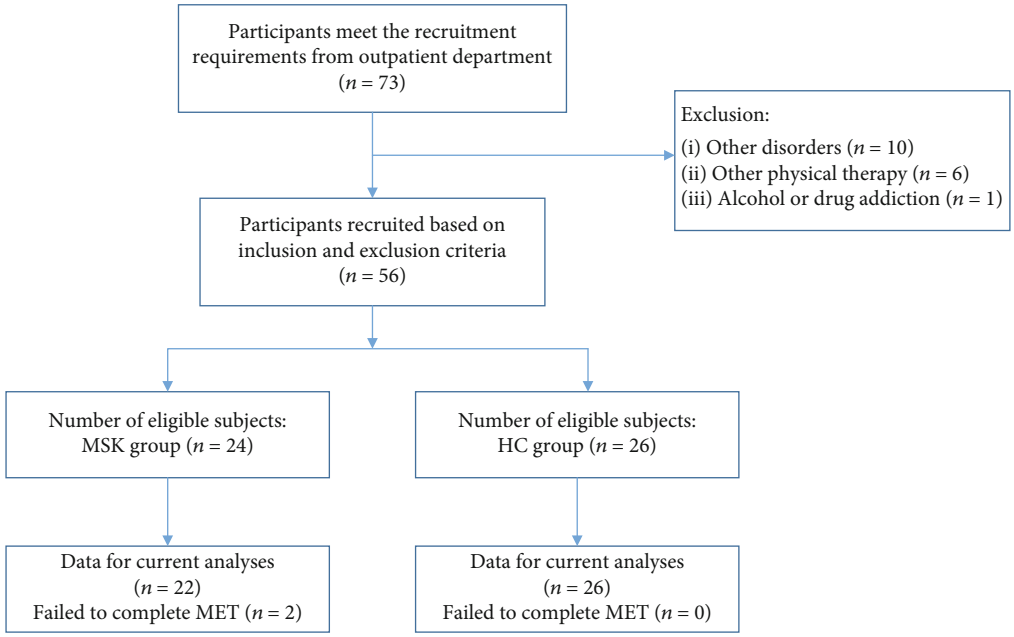

Figure 1: The flow chart of the patients.

Table 1: Anatomical pain sites of the chronic musculoskeletal pain (MSK pain) patients.

Subject number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Shoulder									√	√			√	√	√	√	√					
Leg	√	√	√	√	√	√	√	√	√		√	√							√	√	√	√
Arm	√		√		√	√	√	√		√			√	√	√							√
Back	√	√			√				√		√	√			√	√	√	√	√			

"√": site of chronic musculoskeletal pain.

a more reliable and valid index of an individual's pain experience than other self-reported measures [27]. Multidimensional assessments of pain can reduce potential errors associated with assessment tools.

2.4. Empathy Ability. First, participants' trait cognitive empathy and trait emotional empathy were measured using the IRI, which has four subscales: the perspective-taking and fantasy subscales represent cognitive empathy, while the empathic concern and personal distress subscales represent emotional empathy [28, 29]. Second, as the MET has higher ecological validity for assessing cognitive empathy and emotional empathy than self-reported questionnaires [30], the Chinese version of this task (MET-C) was also used. It involves 40 pictures of people in various emotional states (20 positive and 20 negative emotional valence pictures). After seeing each picture, participants were asked to respond to three questions. First, to assess cognitive empathy accuracy and task completion time, for each picture, they were presented with four words describing four emotions and were asked to select the one that best fits the picture. Next, to assess emotional empathy, participants were asked "How calm/aroused does this picture make you feel?" (indirect emotional empathy) and "How concerned are you for this person?" (direct emotional empathy) on a scale of 0 (not at all) to 9 (very much). The procedures followed those set out by Wu et al. [30] (Figure 2).

2.5. Statistical Analyses. Data were analyzed using STATA software version 15.1 (Stata Corporation, USA). The chronic MSK pain and HC groups were compared using the independent-samples t-test or Pearson's chi-square test, as appropriate. To assess the between-group differences in empathy in the positive or negative emotional valence conditions, repeated-measures analysis of variance (ANOVA), followed by post hoc Bonferroni tests, was used. Pearson correlation analyses were also used to assess associations between empathy (i.e., cognitive empathy accuracy, based on the MET-C) and other variables (pain intensity (SF-MPQ), pain duration, positive/negative emotion (PANAS), sleep quality (PSQI), age, education level, or MET-C task completion time). Multivariate stepwise linear regression was used to assess whether pain factors (SF-MPQ and pain duration) and demographic factors (age and education level) can predict empathy (i.e., cognitive empathy accuracy, based on the MET-C). Data were inspected for normality using the Shapiro–Francia test. Two-tailed P value < 0.05 was considered significant.

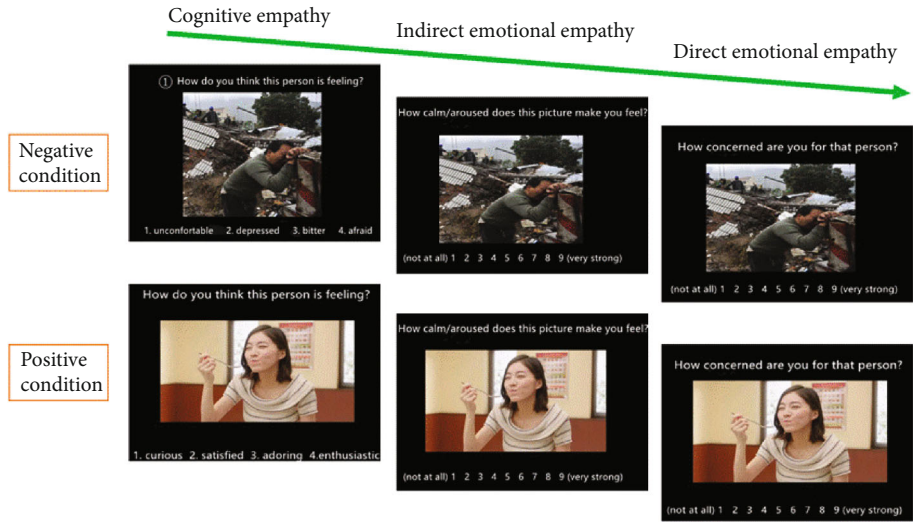

Figure 2: Example items of the MET-C.

3. Results

3.1. Demographic and Clinical Characteristics. The detailed demographic and clinical information of the participants is shown in Table 2. There were no significant differences between the chronic MSK pain and HC groups in age ($P = 0.128$), education level ($P = 0.102$), gender ($P = 0.532$), or handedness ($P = 0.272$), based on independent-samples t-tests and chi-square tests. Regarding clinical characteristics, the chronic MSK pain group had poorer sleep quality than the HC group ($P = 0.014$), while no significant difference was found in positive and negative emotion (positive: $P = 0.351$; negative: $P = 0.058$) or in depression ($P = 0.122$), anxiety ($P = 0.087$), and stress ($P = 0.536$). The Shapiro–Francia test showed that the mean cognitive empathy accuracy (HC group: $P = 0.033$; chronic MSK pain group: $P = 0.005$) and accuracy in positive (HC group: $P = 0.084$; chronic MSK pain group: $P = 0.202$) and negative (HC group: $P = 0.400$; chronic MSK pain group: $P = 0.008$) emotion conditions had nonnormal distributions, while the squared mean cognitive empathy accuracy (HC group: $P = 0.258$; chronic MSK pain group: $P = 0.068$) and squared mean accuracy in positive (HC group: $P = 0.448$; chronic MSK pain group: $P = 0.678$) and negative (HC group: $P = 0.738$; chronic MSK pain group: $P = 0.153$) emotion conditions had normal distributions. Hence, we used the squared value in the analysis.

3.2. Inconsistency between IRI and MET-C. There were no significant differences between the chronic MSK pain and HC groups in IRI trait empathy (disposition to empathic responsiveness according to a self-reported questionnaire; Table 3), comprising mean self-reported cognitive empathy (perspective-taking: Student's $t = 0.442$, $P = 0.660$; fantasy: Student's $t = 0.282$, $P = 0.779$) and mean self-reported emotional empathy (empathic concern: Student's $t = -0.039$, $P = 0.969$; personal distress: Student's $t = -0.058$, $P = 0.954$). Similarly, according to the MET-C, there were no significant differences in either the mean indirect emotional empathy (Figure 3(a); Student's $t = -1.472$, $P = 0.148$) or mean direct emotional empathy (Figure 3(b); Student's $t = 1.345$, $P = 0.185$). However, the MET-C revealed impaired cognitive empathy in chronic MSK pain patients compared to HCs: the chronic MSK pain group had a lower squared cognitive empathy accuracy (Figure 3(c); Student's $t = -2.119$, $P = 0.040$) and a longer task completion time (Figure 3(d); Student's $t = 3.382$, $P = 0.002$). The repeated-measures ANOVA indicated a significant main effect of group ($F = 4.614$, $P = 0.037$, $\eta^2 = 0.091$), emotion valence ($F = 5.660$, $P = 0.022$, $\eta^2 = 0.110$), and interaction term ($F = 4.254$, $P = 0.045$, $\eta^2 = 0.085$). Post hoc analysis showed that the impaired cognitive empathy was pronounced in the positive emotion condition ($F = 9.105$, $P = 0.004$, $\eta^2 = 0.165$) but there was no difference in cognitive empathy in the negative emotion condition ($F = 0.055$, $P = 0.816$, $\eta^2 = 0.001$; Figure 4).

3.3. Correlation between Pain and Empathy. Pearson correlation analysis showed a significant negative correlation between pain intensity (SF-MPQ) and squared cognitive empathy accuracy (MET-C; $r = -0.606$, $P = 0.003$), but not between pain duration and squared cognitive empathy accuracy (Table 4). Additionally, stepwise multivariate linear regression showed that only pain intensity (SF-MPQ) was significantly associated with squared cognitive empathy accuracy (adjusted $R^2 = 0.335$, $P = 0.003$, $b = -0.024$; Figure 5(a)), but pain duration and other demographics were not (Figure 5(b)). There were also no correlations between squared cognitive empathy accuracy (either mean accuracy or accuracy in the positive or negative conditions) and positive/negative emotion (PANAS), sleep quality (PSQI), age, or education level (Table 4).

4. Discussion

This study revealed the impaired cognitive empathy (i.e., lower squared cognitive empathy accuracy and longer task completion time based on the MET-C) in chronic MSK pain patients compared to HCs. This is consistent with previous research on chronic MSK pain, which found impaired

Table 2: Demographic and psychological characteristics of chronic musculoskeletal pain (MSK pain) patients and healthy controls (HCs).

	MSK pain ($n = 22$)	HC ($n = 26$)	t (χ^2)	P
Age	44.41 ± 7.94	40.08 ± 10.86	1.550	0.128
Education years	9.41 ± 4.08	11.64 ± 4.99	-1.650	0.102
Gender (male/female)	17/5	18/8	0.3903	0.532
Handedness (left/right)	0/22	1/25	1.2070	0.272
NRS	5.64 ± 2.81	0	NA	NA
SF-MPQ	7.36 ± 4.81	0.04 ± 0.200	NA	NA
Pain time (months)	22.21 ± 33.62	0	NA	NA
PSQI	6.91 ± 4.80	4.19 ± 2.30	2.550	0.014*
Positive emotion	19.86 ± 6.94	21.69 ± 6.49	-0.950	0.351
Negative emotion	18.36 ± 7.14	15.19 ± 3.94	1.950	0.058
Depression	11.45 ± 3.46	10.04 ± 2.76	1.600	0.122
Anxiety	11.59 ± 3.75	10.04 ± 2.32	1.750	0.087
Stress	13.23 ± 3.57	12.62 ± 3.23	0.600	0.536

P represents level of significance from independent-samples t-test and chi-square as appropriate. NRS: Numerical Rating Scale; SF-MPQ: Short-Form McGill Pain Questionnaire; PSQI: Pittsburgh Sleep Quality Index. *$P < 0.05$; NA: not applicable.

Table 3: IRI fantasy (FS), perspective taking (PT), empathic concern (EC), and personal distress (PD) subscale scores in chronic musculoskeletal pain (MSK pain) and healthy control (HC) groups. The perspective-taking and fantasy subscales represent self-report cognitive empathy, while the empathic concern and personal distress subscales represent self-report emotional empathy.

		MSK pain (n = 22)	HC (n = 26)	t	P
Self-repot cognitive empathy	PT	21.86	21.27	0.442	0.660
	FS	18.14	17.77	0.282	0.779
Self-repot emotional empathy	EC	23.00	23.04	-0.039	0.969
	PD	19.50	19.58	-0.058	0.954

No significant difference between groups was found in IRI.

empathy (as measured by the Basic Empathy Scale in Adults) in patients with chronic low back pain [17]. However, we found that the chronic MSK pain patients' emotional empathy was not impaired. This inconsistency between cognitive empathy and emotional empathy in patients has also been reported in adults with Asperger's syndrome (one of the primary symptoms: impaired social interaction), indicating that these individuals with chronic MSK pain can also be confused by others' emotions [21]. Besides, impaired cognitive empathy with good emotional empathy was thought to be a psychotic symptom, which in chronic MSK pain can also result in interpersonal problems and social stigmatization for patients [31]. In summary, using a behavior task (i.e., the MET-C), we found that impaired cognitive empathy (without impaired emotional empathy) was evident in the chronic MSK pain patients, which may lead to a decline in prosocial behavior.

In addition, the finding regarding the impaired cognitive empathy in chronic MSK pain patients was also supported by the correlation analysis, which demonstrated that pain intensity (SF-MPQ) was negatively correlated with cognitive empathy accuracy, as in a previous study [18]. However, there was no correlation between pain duration and cognitive empathy accuracy. This means that even in the early stage of chronic MSK pain, cognitive empathy possibly has declined to a low level, and pain intensity rather than pain duration is associated with the degree of cognitive empathy impairment. This result highlights the importance of identifying impairments in empathy with a suitable assessment tool in the early stage of chronic pain. Compared to the subjective NRS, the SF-MPQ has more descriptive details about types of pain sensations (e.g., throbbing, shooting, stabbing, and fear), and it is a more objective multidimensional measure [32]. Based on the correlation between pain intensity (SF-MPQ) and empathy (cognitive empathy accuracy, based on the MET-C), it might indicate that the SF-MPQ provided additional information about the level of ability to recognize emotions.

Furthermore, the results revealed that cognitive empathy impairment was linked to emotional valence. The chronic MSK pain patients did not report an intensely subjective experience of emotion (i.e., PANAS) in either the positive or negative emotion conditions. Patients had the same cognitive empathy accuracy as HCs (i.e., they recognized/understood the emotions) in the negative emotion condition, and the chronic MSK pain and HC groups had the same direct and indirect emotional empathy in the negative emotion condition. In contrast, patients had significantly impaired

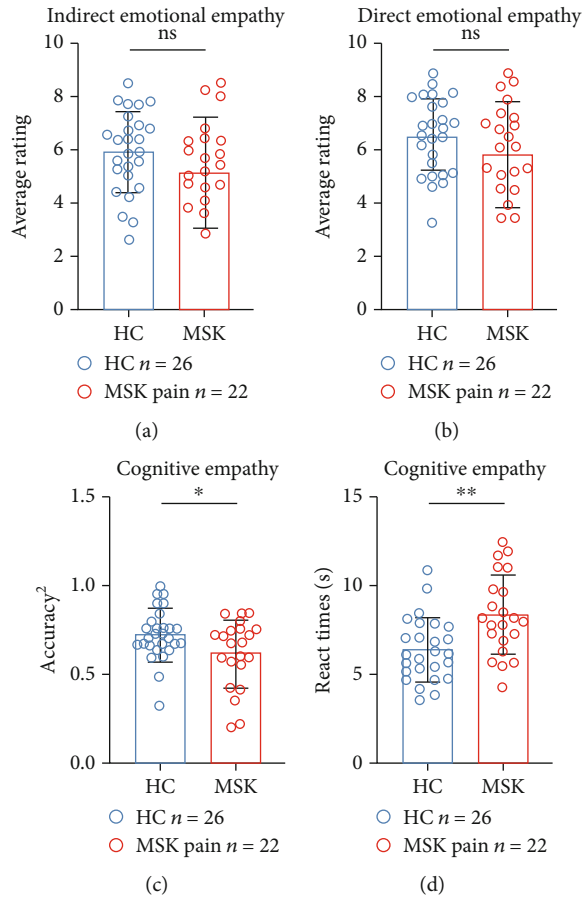

Figure 3: Comparisons of the indirect emotional empathy score (a), direct emotional empathy score (b), squared cognitive empathy accuracy (c), and task completion time for cognitive empathy section (d) between chronic musculoskeletal pain (MSK pain) and healthy control (HC) groups. ns: no significant; $^*P < 0.05$; $^{**}P < 0.01$.

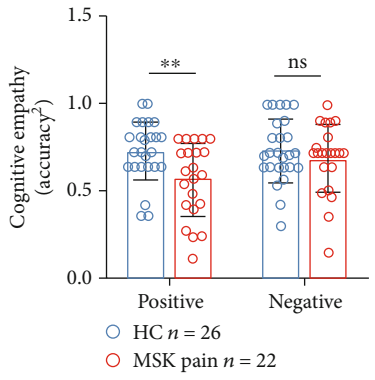

Figure 4: Comparisons of squared cognitive empathy accuracy between chronic musculoskeletal pain (MSK pain) and healthy control (HC) groups in positive/negative emotional valence conditions. In the positive condition, the squared cognitive empathy accuracy was significantly lower in the MSK pain group than the HC group, while there was no significant difference in the negative condition. ns: no significant; $^{**}P < 0.01$.

cognitive empathy accuracy in the positive emotion condition compared to HCs, though these groups had the same direct and indirect emotional empathy in the positive emotion condition. This might be caused by compensation for the impaired empathetic recognition (i.e., impaired cognitive empathy accuracy) in the positive emotion condition [33]. From this, it can be seen that accurately detecting the levels of empathy ability and pain requires that appropriate comprehensive assessment methods are used.

Notably, there were no significant between-group differences in IRI, a typical self-reported scale for assessing cognitive empathy and emotional empathy, despite there being a significant difference according to the MET-C. Thus, by utilizing the MET-C, which involves visual stimulation encompassing different emotions, we highlighted the applicability of a more objective empathy assessment in chronic pain patients.

The PANAS scores and even the DASS-21 scores did not differ significantly between groups, which conflicts with previous studies demonstrating significant increases in depression and stress in chronic MSK pain patients [24, 34]. This may be readily explained by the findings of Cruz-Almeida et al. of large individual differences in pain and psychological function [35]. Analysis of chronic pain patient subgroups with specific sets of clinical characteristics is needed to fully explore differences within the chronic pain patient population. In addition, some of the previous research involved hospital patients with severe illness, with more psychological

Table 4: Correlations of MET-C performance, including accuracy (mean accuracy and accuracy in positive/negative conditions) and task completion time, with positive/negative experienced emotion (PANAS) and other variables (age, education duration, PSQI, pain intensity, and pain duration).

	Age	Education years	PSQI	Positive PANAS	Negative PANAS	SF-MPQ	Pain time	Completion time	Mean accuracy^2	Positive accuracy^2	Negative accuracy^2
Age	1										
Education years	-0.601**	1									
PSQI	-0.032	0.536*	1								
Positive PANAS	-0.031	-0.070	-0.095	1							
Negative PANAS	-0.233	0.134	0.224	0.390	1						
SF-MPQ	-0.109	0.089	0.275	-0.343	0.400	1					
Pain time	0.102	-0.017	0.186	-0.209	-0.044	0.090	1				
Completion time	0.497	-0.416	0.101	-0.002	0.024	0.184	-0.037	1			
Mean accuracy^2	-0.237	0.286	0.036	0.341	-0.083	-0.606**	-0.374	-0.436*	1		
Positive accuracy^2	-0.373	0.299	0.075	0.275	-0.074	-0.562**	-0.331	-0.525*	0.926***	1	
Negative accuracy^2	-0.031	0.205	-0.019	0.353	-0.060	-0.547**	-0.362	-0.272	0.901***	0.673***	1

Values reported are Pearson correlation coefficients. PSQI: Pittsburgh Sleep Quality Index; PANAS: Positive and Negative Affectivity Scale; SF-MPQ: Short-Form McGill Pain Questionnaire. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

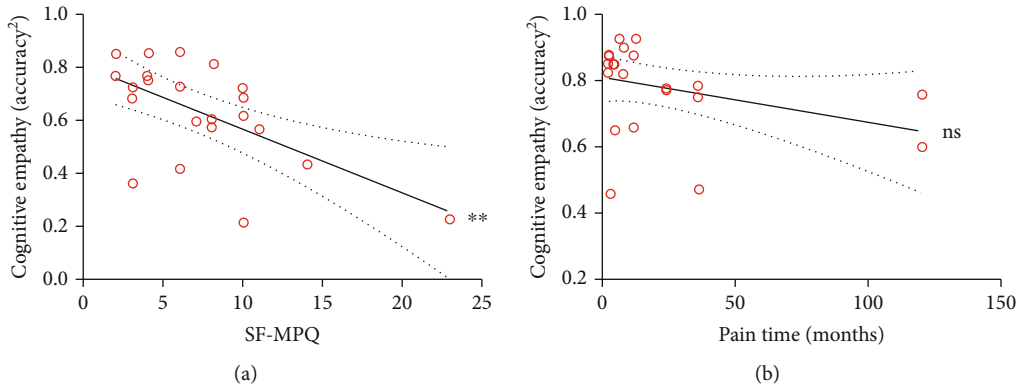

Figure 5: Correlations of SF-MPQ with squared cognitive empathy accuracy and pain duration. (a) Positive correlation between SF-MPQ and squared cognitive empathy accuracy. (b) No correlation between pain duration and cognitive empathy accuracy. The thick line indicates the regression line, and the dotted arcs indicate the confidence limits. ns: no significant; $^{**}P < 0.01$.

and somatic symptoms and poorer quality of life. The difference in clinical status and treatment settings may explain the inconsistencies with previous research [36]. Changes in a patient's normal living environment (including changes in social and affiliative behaviors) are not conducive to studying empathy or reflecting the actual patient situation. We recruited chronic MSK pain outpatients (who were not hospitalized and thus had similar social circumstances to the HCs) to make the comparison more reliable; thus, the differences observed in our data mostly reflect the presence of chronic MSK pain rather than other factors. Furthermore, another study suggested that chronic pain in different body regions might be reflective of different brain signatures [37], which may also help to explain the inconsistencies between our results (involving no significant differences in PANAS or DASS-21 between chronic MSK pain patients and HCs) and the previous results. As our findings highlight that, for chronic pain, early detection of impaired empathy and preventive strategies are particularly important, our findings on empathy may provide value for ambulatory chronic pain patients.

Our findings also concur with neurological research on chronic pain. Regarding general MSK chronic pain, the main dysfunctional cortices are the cingulate, prefrontal, and primary/secondary somatosensory cortices [38], and these impaired brain areas are also involved in empathic processing (e.g., the medial/lateral prefrontal cortex conceivably mediates empathy by processing information and action-relevant stimuli [39], and the primary somatosensory cortex plays an important role in both actual pain perception and social recognition [40]). These facts provide a hint about the connectedness between empathy and chronic MSK pain and a potential intervention target for impaired empathy in chronic MSK pain patients.

Previous research on the relationship between chronic pain and empathy has focused on the effect of observers' empathy for chronic pain patients rather than the empathy ability of patients themselves [41]. Various literatures on pain and empathy have demonstrated that the failure of surgery was more likely attributed to patient's psychological dysfunction [42]; the effect of intervening was correlated with the empathy they have perceived [43]. However, these models have focused on the empathy of observers rather than the patients', while trying to control relations with others to improve patient's symptoms seems unlikely. Considering the model of pain and empathy and the fact that patients' own empathy ability is highly positively correlated with their social support system [44], our results potentially provide a novel approach for the treatment of chronic MSK pain. That said, focusing on patients' empathy ability makes chronic MSK pain treatment through controlling social factors possible and feasible.

There are several limitations to our study. First, the results are limited due to the relatively small sample size and cross-sectional design. Although an association was identified, we cannot prove causality regarding the effect of empathy on chronic MSK pain without longitudinal follow-up. Second, a single behavior task (MET-C) was utilized to assess empathy. The lack of ancillary neurological testing (involving neuroimaging or electroencephalography) meant that the underlying mechanisms could not be fully explored. Third, other tools that assess basic empathy-related functions (such as facial emotion recognition, emotion-related memory, and emotion-related decision making) should be used to investigate how cognitive empathy impairment occurs.

5. Conclusion

Collectively, our results to date indicated that chronic MSK pain just in outpatients could lead to social dysfunction, suggesting the importance to evaluate the empathetic function of this disease with suitable tools at an early age. These findings contribute to our understanding of the impaired empathy in chronic MSK pain patients and give doctors and physicians a starting point to consider the social and psychological factors in clinical decisions of chronic MSK pain, complementing current research and developing promising interventions.

Data Availability

The original data and related materials of this study can be accessed from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

HBZ and QL participated in the data collection. HBZ, HO, LZ, and ZFY conducted the data analyses and wrote the original manuscript. YS, CH, XL, and DHM reviewed the manuscript. YS, ZFY, CH, and HBZ conceived the idea and designed the experiment. YS, CH, and XL provide the funding. Hang-Bin Zhang, Hang Ou, Dian-Huai Meng, and Qian Lu contributed equally to this work.

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Research Article

Differential Proteomic Analysis of the Hippocampus in Rats with Neuropathic Pain to Investigate the Use of Electroacupuncture in Relieving Mechanical Allodynia and Cognitive Decline

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Abnormal changes in hippocampal function and neuroplasticity are involved in neuropathic pain, which induces hyperalgesia and learning and memory deficits. Previous studies from our group have shown that electroacupuncture at Huantiao (GB30) and Yanglingquan (GB34) has an obvious analgesic effect on neuropathic pain. However, the central regulatory mechanism occurring in the hippocampus remains to be investigated. In this study, behavioral and proteomic analyses were performed to identify differentially expressed hippocampal proteins involved in electroacupuncture-induced analgesia. Our results showed both upregulated (TMEM126A, RDH13, and Luc7L) and downregulated proteins (Mettl7A, GGA1 RTKN, RSBN1, and CDKN1B). Further protein verification revealed for the first time that hippocampal TMEM126A plays an important anti-inflammatory role in the treatment of neuralgia by electroacupuncture.

1. Introduction

Cognitive impairment is commonly associated with the experience of pain, with approximately 73%–81% of patients with chronic pain suffering from a memory deficit [1, 2]. Recent clinical studies have reported objective cognitive impairments in patients with peripheral neuropathic pain [3, 4]. Several studies have provided evidence suggesting that structural and functional abnormalities in the hippocampus underlie the cognitive deficits associated with neuropathic pain [5–8]. The hippocampus receives complex integrated sensory and cognitive information, including pain signals. The changes of some gene and proteins such as the upregulation of TNF-α, IL-1, and MCP-1 in the hippocampus play key roles in the neuropathic pain accompanied with cognitive impairment in rodents [9, 10]. However, the hippocampal proteins and their related signaling pathways involved in neuropathic pain still remain largely elusive.

Electroacupuncture (EA) at acupoints Huantiao (GB30) and Yanglingquan (GB34) is effective in relieving pain sensitization and cognitive decline associated with neuropathic pain in humans and rodents [11–13]. Spinal opioids, serotonin, norepinephrine, amino acids, and glial cells/cytokines are the primary mediators of EA-induced analgesia of neuropathic pain [14]. Recent studies have focused on the role of hippocampal functional changes in the beneficial effect of EA on the pain condition. Regulation of the hippocampal proteins related to amino acid metabolism and activation of the MAPK signaling pathway is involved in the analgesic effect of EA in neuropathic pain [15, 16]. However, few studies to date have focused on the role of the molecular function of the hippocampus in EA-induced analgesia and cognitive improvement.

Proteomics analysis provides a valuable strategy for exploring the pathogenesis of pain mellitus, as well as therapeutic targets in this condition. We hypothesize that some key genes or proteins in the hippocampus play an essential role in the neuropathic pain with EA treatment. Based on the prior work outlined above, this study was aimed at exploring the molecular mechanisms underlying

EA-induced analgesia in the hippocampus using proteomic and behavioral analyses.

2. Materials and Methods

2.1. Animals and Materials. Male Sprague-Dawley (SD) rats (8–10 weeks, 200–220 g) were obtained from the Shanghai Slack Laboratory Animal Co. (Shanghai, China). The rats were housed under stable temperature conditions (22°C) and a 12/12 h light/dark cycle with free access to food and water at the Experimental Animal Center of Fujian University of Chinese Medicine. SD rats were randomly divided into a Sham operation group (Sham), a spared nerve injury model group (SNI), and an electroacupuncture group (EA). Ten animals per group underwent behavioral testing and analysis, and four animals per group were used for Enzyme-Linked Immunosorbent Assay (ELISA) and western blot testing after all behavioral testing. All animal experimental protocols were approved by the Animal Ethics Committee of Fujian University of Traditional Chinese Medicine (No. FJTCM2019-006). All rats were humanely sacrificed according to the care guidelines. The rats were anesthetized and euthanized with more isoflurane to minimize the pain experienced by the animals.

The following reagents and instruments were used: von Frey filament (North Medical, CA, USA); acupuncture needle, 0.30 mm × 25 mm (Suzhou Medical Supplies Factory, China); Tmem126a ELISA kit (Wuhan Boster Biological Technology, China); TMEM126A rabbit pAb (A12823; ABclonal Technology, China); and GAPDH goat pAb (ab9483; Abcam, USA).

2.2. Spared Nerve Injury-Induced Neuropathic Pain Model and EA Stimulation. The spared nerve injury of sciatic nerve was performed under anesthesia according to the procedures described previously [17]. In the SNI and EA groups, the right sciatic nerve branches of rats were exposed and performed selective cutting, or ligation. Briefly, the common peroneal and the tibial nerves were separated, tightly ligated with 5-0 silk, and transected distal to the ligation. A 2 mm length of each nerve was removed, while preserving the integrity of the sural nerve. In the Sham operation group, only the sciatic nerve branch was exposed, without performing any nerve severance or ligation. Rats were monitored for any sign of infection or distress after surgery.

Seven days after spared nerve injury surgery, EA group rats were loosely fixed on a wooden stand that permitted free movement of their head and limbs. The acupoints Huantiao (GB30) and Yanglingquan (GB34) on the right side were selected for acupuncture and electrical stimulation. In the rat, GB30 is located at the junction of the lateral third and medial two-thirds of the line connecting the prominence of greater trochanter of the femur with the sacral hiatus, while GB34 is located in the depression anterior and distal to the head of the fibula [18, 19]. EA stimulation was performed for 30 min, once a day for three consecutive weeks. Needles inserted at GB30 and GB34 were connected to a G6805-1A multifunctional EA apparatus (Shanghai Medical Electronic Apparatus Company, Shanghai, China), using a stimulation intensity of 1 mA and a frequency of 2 Hz. The Sham operation group and model group underwent the experimental protocol at the same time each day.

2.3. Behavioral Testing. The mechanical paw withdrawal threshold (PWT) was assessed on day 0, 7, 14, 21, and 28 postsurgery after EA stimulation using von Frey filaments. According to the up-down method, von Frey filaments of different intensities (0.6 g, 1.0 g, 1.4 g, 2.0 g, 4.0 g, 6.0 g, 8.0 g, 15.0 g, and 26.0 g) were used to determine the 50% PWT in rats. A quick paw withdrawal or licking of the paw in response to the stimulus was considered to be a positive response. The protocol for assessing the PWT was based on the one described in our previous study [20].

The novel-object recognition test was performed in a Plexiglas box (60 mm × 60 mm × 50 mm) on day 28 postsurgery. After habituation, on day 1, the rats were placed in the box for 20 min without objects being presented. Four hours later, the rats were placed in the box again and allowed to explore objects A and B for 5 min. After a retention interval of 24 h, a cognitive test was performed in the same box by replacing object B with a novel object C. The rats were then allowed to explore the objects freely for 5 min. The ratio of the difference between the time spent exploring the familiar object and that spent exploring the novel object over the total time spent exploring both objects was used to evaluate cognitive function.

2.4. Proteomic Detection and Analysis

2.4.1. Protein Extraction and Trypsin Digestion. All methods of proteomic detection and analysis were according to our team's previous research as follows [21].

Proteins were extracted from the hippocampal tissue samples in lysis buffer (8 M urea, 1% protease inhibitor) using a high-intensity ultrasonic processor. Remaining debris was removed by centrifugation at 12,000 × g for 10 min at 4°C. The protein concentration was detected with a Bicinchoninic Acid (BAC) kit.

For trypsin digestion, protein solution was reduced with dithiothreitol for 30 min at 56°C, then incubated with iodoacetamide for 15 min at 37°C in darkness. Secondly, protein samples were diluted with millimolar Triethylammonium bicarbonate (TEAB), until the urea concentration was < 2 M. Finally, trypsin was added at a mass ratio (trypsin : protein) of 1 : 50 for the first digestion at 37°C overnight, then at a ratio of 1 : 100 for the second digestion.

2.4.2. Tandem Mass Tag (TMT) Labeling and LC-MS/MS Analysis. After trypsin digestion, peptides were desalted and vacuum-dried with the Strata X C18 SPE column. Peptides were reconstituted in 0.5 M TEAB and marked according to the TMT kit protocols.

Separated peptides were subjected to sodium/iodide symporter sources. Tandem mass spectrometry (MS/MS) was performed using a Q Exactive Plus (Thermo) instrument, coupled online to an ultraperformance liquid chromatography system.

Using an electrospray voltage of 2.0 kV, intact peptides were then detected in the Orbitrap at 70,000 mass resolution. The primary MS scan range was 350–1,600 m/z. Collected

data were processed using a data-dependent scanning program (DDA). Automatic gain control was set at 50,000, with a signal threshold of 5,000 ions/s, maximum time of 200 s, and dynamic exclusion time of the tandem mass scan of 15 s, to avoid repeated scanning of precursor ions.

2.4.3. Database Search. MS/MS data were analyzed using the MaxQuant search engine (v.1.5.2.8), with the following parameters: rat UniProt was first screened; then, a reverse library was added to calculate the false-positive ratio (FPR); trypsin/P was specified as the cleavage enzyme, allowing up to two missed cleavages; the minimum peptide length was 7 amino acid residues; the maximum number of peptide modifications was 5; the mass tolerance for the primary precursor ions search was 20 ppm, while that for the main search was 5 ppm; the mass tolerance for fragment ions was 0.02 Da; the quantitative method was set to TMT-10plex; and the FPR for peptide-spectrum match identification was set to 1%.

2.4.4. Bioinformatic Annotation. Gene Ontology (GO) annotations were derived from the UniProt-GOA database (http://www.ebi.ac.uk/GOA/). InterProScan soft was used to analyze the following GO categories: molecular function, cellular component, and biological process. The KEGG online service tools were used to annotate protein descriptions, which were matched to their corresponding pathways using the KEGG mapper. WoLFPSort (https://wolfpsort.hgc.jp/) was used to investigate subcellular localization. KEGG database pathway enrichment analysis was conducted using the two-tailed Fisher exact test. Pathways were classified according to the KEGG website.

Further cluster analysis of functional enrichment was conducted to explore potential connections and differences in specific functions. First, data for all GO categories were collected after enrichment, sorted according to their P values, then filtered to obtain the data for categories with $P < 0.05$. This filtered P value matrix was transformed using the function $x = -\log_{10}(P)$. Finally, the x values for each functional category were z-transformed. Cluster membership was visualized using a heat map.

2.5. ELISA Testing. Concentrations of Tmem126a were analyzed using an ELISA kit according to the manufacturer's instructions. Briefly, 100 μl, respectively, of standard and diluted samples in duplicated wells was incubated at room temperature for 2 h. After the wells were washed 5 times with 1× wash solution, 100 μl enzyme conjugate reagent was added to each well and the wells were incubated for 2 h at room temperature. 100 μl substrate solution was then added, and after 30 min incubation, 100 μl of stop solution was added to terminate the reaction. The resultant color was assayed using a microtiter plate reader.

2.6. Western Blot Testing. After spared nerve injury surgery, rats ($n = 4$ per group) were sacrificed on day 28 in order to perform analyses of hippocampal proteins. Protein extraction, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and western blotting analyses were performed. The protein was extracted using homogenization in SDS sample buffer, followed by centrifugation at 12,000 × g for 20 min. The protein concentration of the supernatant was determined using the BCA Protein Assay Kit (Pierce, Rockford, USA), and 60 μg of protein was loaded into each lane of the 10% SDS–PAGE gel. The membrane was blocked overnight using 5% bovine serum albumin in TBS-T. The blot was then probed using the following primary antibodies: rabbit anti-TMEM126A antibody (1 : 500) and goat anti-GAPDH (1 : 1,000) overnight at 4°C. Then, the blot was incubated with HRP-anti-rabbit/goat (1 : 2,000; Santa Cruz, CA, USA) antibody for 1 h at room temperature. The blots were incubated in ECL (Pierce) solution for 3 min and then exposed onto Kodak X-OMAT AR Film (Eastman Kodak, Rochester, USA) for 1.5 min. Densitometric analysis of the TMEM126A bands was performed using Syngene software (Gene Gnome, Syngene, MD, USA).

2.7. Statistical Analysis. All data are presented as the mean ± standard deviation (SD). The three experimental groups were analyzed using the one- and two-way Analysis of Variance (ANOVA) and post hoc Tukey tests in SPSS 21.0 (SPSS, Armonk, NY, USA). Graphs were generated using GraphPad Prism 7.0 software.

3. Results

3.1. EA Abolishes Mechanical Pain Sensitivity and Memory Deficits Induced by Neuropathic Pain. As shown in Figure 1(a), compared with the SNI model group, the EA group showed a significantly increased mechanical pain threshold on days 7, 14, and 21 of neuropathic pain ($P < 0.001$). On day 28 of spared nerve injury, as shown in Figure 1(b), the novel object recognition index significantly decreased in the SNI rats ($P < 0.001$). Compared with the SNI model group, the novel object recognition index was significantly increased in the EA group ($P < 0.001$).

3.2. Hippocampal Proteomic Changes in SNI Rats Treated with EA. Compared with the Sham operation group, 16 proteins were upregulated and 11 proteins were downregulated in the hippocampus of the SNI model group. Compared with the SNI model group, 17 proteins were upregulated and 36 proteins were downregulated in the EA group (Figures 2(a) – 2(c)).

Among the 27 regulatory proteins induced by spared nerve injury, the most significantly upregulated or downregulated proteins, respectively, were transmembrane protein 126A (TMEM126A) and excitatory amino acid transporter 2 (SLC1A2/EAAT2), which have mainly been associated with persistent pain, cognitive impairment, and immune-inflammatory regulation (Table 1). ELISA and western blot results showed that after continuous EA, TMEM126A expression was significantly decreased in the SNI group compared with the Sham group, while TMEM126A expression was significantly upregulated, consistent with the proteomic results (Figures 2(d) and 2(e)).

Further proteomic detection and analysis showed that spared nerve injury-induced downregulated proteins such as TMEM126A, RDH13, and Luc71 were upregulated in the EA group. In addition, spared nerve injury-induced

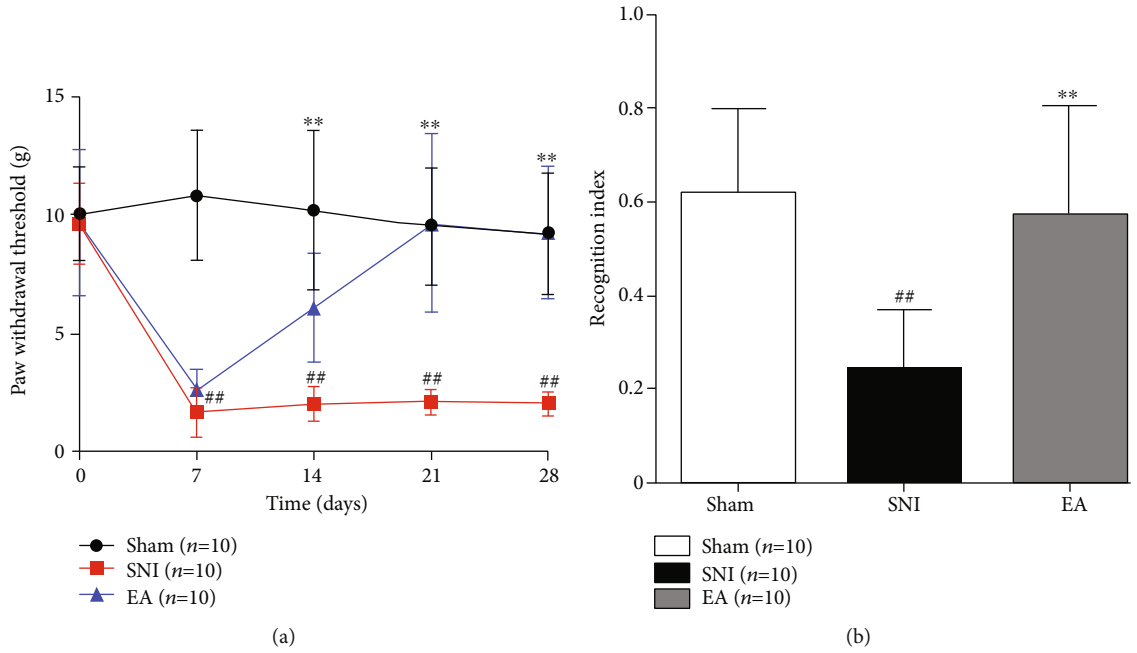

FIGURE 1: (a) Paw withdrawal in response to mechanical pain varies among the Sham, SNI, and EA groups (two-way repeated ANOVA, $F = 205.036$, $P < 0.001$; post-hoc Tukey test, Sham compared with SNI, $^{\#\#}P < 0.001$; EA compared with SNI, $^{**}P < 0.001$). (b) Significant changes of recognition index detected with new object recognition behavior test in different groups (one way ANOVA, $F = 12.433$, $P < 0.001$; post hoc Tukey test, Sham compared with SNI, $^{\#\#}P < 0.001$; EA compared with SNI, $^{**}P < 0.001$).

upregulated proteins such as MettL7A, GGA1, RTKN, rsBN1, and CDKN1B were significantly downregulated after EA treatment (Table 2).

3.3. Analysis of Biological Process, Cell Component, and Molecular Function in SNI and EA Group Rats. In terms of biological process analysis, the top four regulated processes, respectively, were cellular process, single-organism process, metabolic process, and biological regulation. For the cell component analysis, the most common proteins were mainly associated with the cell and organelle components. The molecular function analysis suggested that the most common molecular functions were binding and catalytic activity (Figures 3(a) and 3(b)).

According to the subcellular structural localization analysis, the main subcategories (over 20%) for the SNI/Sham proteins were nuclear (33.33%) and extracellular (22.22%), while those for the EA/SNI proteins were cytoplasmic (37.74%) and nuclear (33.96%) (Figures 3(c) and 3(d)).

3.4. Functional Enrichment and Cluster Analysis of the SNI and EA Groups Using KEGG Pathway Analysis. To further explore which pathways were significantly affected by the differentially expressed proteins, the proteins were mapped to the KEGG database and their enrichment levels were calculated using the Fisher exact test P value $[-\log_{10}(P)]$. As shown in Figures 4(a) and 4(b), the KEGG pathway enrichment analysis demonstrated that M/S differentially expressed proteins were mainly enriched in renin–angiotensin system and folate biosynthesis pathways. In contrast, the E/M differentially expressed proteins were mainly enriched in the drug metabolism, cytochrome P450, and metabolism of xenobiotics by cytochrome P450 pathways.

4. Discussion

In our research, we found that 4 weeks of continuous EA treatment in rats significantly increased their mechanical pain threshold and improved cognitive deficits caused by spared nerve injury-induced neuropathic pain. This is the first time that hippocampal proteomic results have been published regarding spared nerve injury-induced neuropathic pain in rats treated with repeated EA stimulation. In total, we quantified 4,804 proteins using the TMT labeling proteomic method. Of these proteins, only 16 were upregulated, while 11 were downregulated during the development of neuropathic pain.

These differentially expressed proteins were mainly involved in the renin–angiotensin system and folate biosynthesis; for example, ACE2 and tryptophan hydroxylase-2 (TPH2), which were downregulated in the neuropathic pain model rats. Recently, some studies have shown that central ACE2 and TPH2 are involved in pain persistence and cognitive decline. ACE2 deficiency has been found to impair cognitive function, increase oxidative stress, and decrease BDNF levels in the hippocampus [29]. The number of ACE2-positive neurons was significantly decreased in diabetic neuropathic pain and is linked to the apoptosis of inhibitory neurons, such as GABAergic interneurons, in the spinal dorsal horn [30]. Tph2 shRNA expression in RVM neurons induced a significant downregulation of Tph2 in the RVM and 5-HT in spinal dorsal horn, attenuating nerve injury-induced allodynia [31].

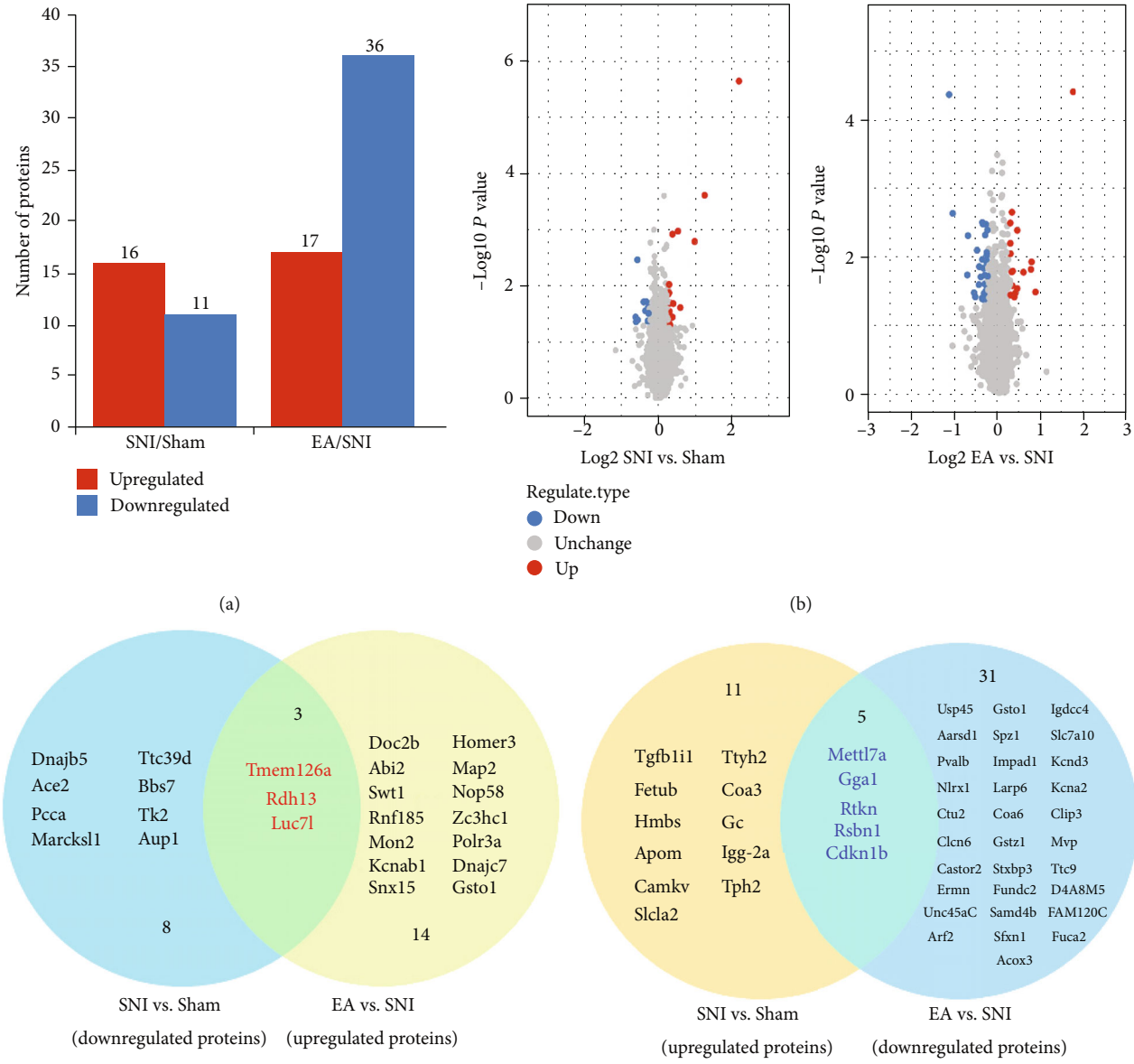

Figure 2: Continued.

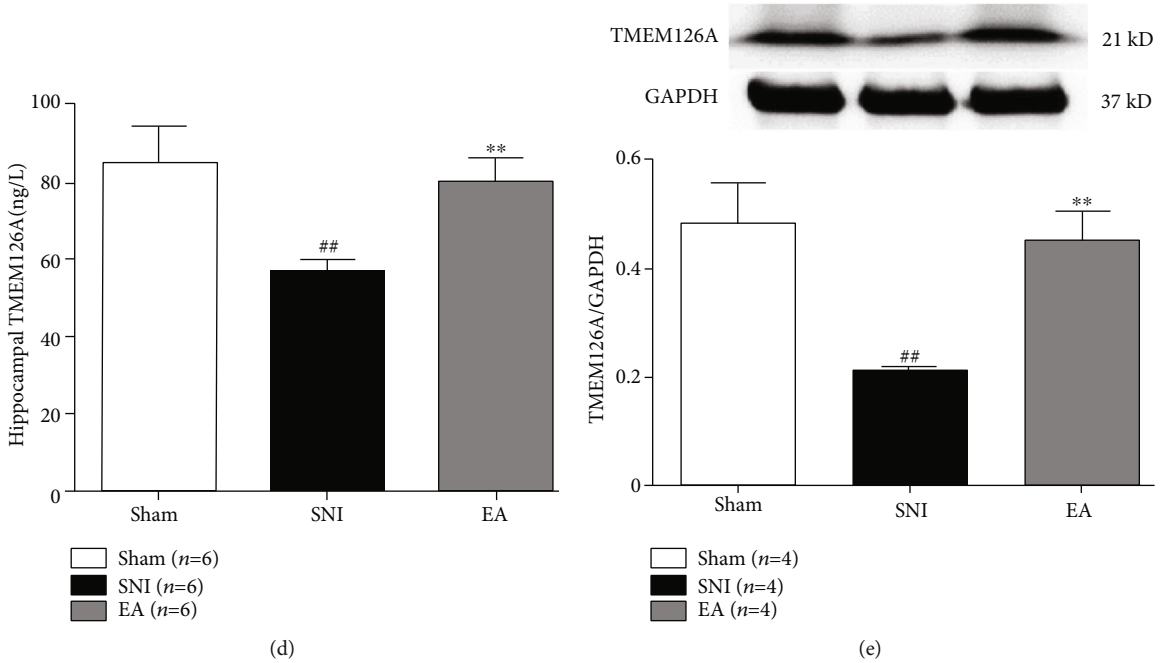

Figure 2: Quantification of differentially expressed proteins in the three groups. (a) Number of differentially expressed protein using a threshold of 1.2-fold (EA group vs. SNI group; SNI group vs. Sham group). (b) Volcanic scatter plot with a threshold of 1.2-fold. (c) Number of EA-regulated SNI-induced differential proteins shown in the Venn diagram using a threshold of 1.2-fold. (d) Hippocampal TMEM126A expression detected with ELISA in the different groups (one-way ANOVA, $F = 33.049$, $P < 0.001$; post hoc Tukey test, Sham group vs. SNI group, $^{\#\#}P < 0.001$; EA group vs. SNI group, $^{**}P < 0.001$; $n = 6$ per group). (e) Hippocampal TMEM126A expression detected via western blot in the different groups (one-way ANOVA, $F = 31.932$, $P < 0.001$; post hoc Tukey test, Sham group vs. SNI group, $^{\#\#}P < 0.001$; EA group vs. SNI group, $^{**}P < 0.001$; $n = 4$ per group).

Table 1: Proteomic information and biological functions of TMEM126 and AEAAT2.

SNI vs. Sham most significant differential proteins	Transmembrane protein 126A	Excitatory amino acid transporters (SLC1A2/EAAT2)
Change ratio	0.64	4.681
P value	0.0016	0.000000212
Subcellular localization	Cytoplasm	Plasma membrane
KEGG orthology	K18157	K05613
Main role of current reports	Immune-inflammatory and tumor metastasis regulation [22–24]	Clearance of spinal glutamate released in pain states; restore hippocampus-dependent memory deficit [25–28]

Table 2: Information of common changed proteins in the three groups.

Protein accession	Protein description	SNI/Sham ratio (down-/upregulated)	*P* value	EA/SNI ratio (up-/downregulated)	*P* value	Gene name
Q5HZA9	Transmembrane protein 126A	0.64	0.0358	1.704	0.0016	Tmem126a
D3ZFR9	Retinol dehydrogenase	0.646	0.0433	1.817	0.0433	Rdh13
G3V9R0	LUC7-like	0.663	0.0033	1.688	0.0034	Luc7l
Q3KRE2	Methyltransferase-like 7A	1.23	0.0281	0.81	0.0334	MettL7A
Q5FVF3	ARF-binding protein 1	1.303	0.0012	0.746	0.02004	GGA1
Q6V7V2	Rhotekin	1.515	0.0241	0.727	0.0141	RTKN
D4A1U7	Round spermatid basic protein 1	1.996	0.0016	0.472	0.0023	Rsbn1
O08769	Cyclin-dependent kinase inhibitor	2.419	0.0002	0.445	0.00004	Cdkn1b

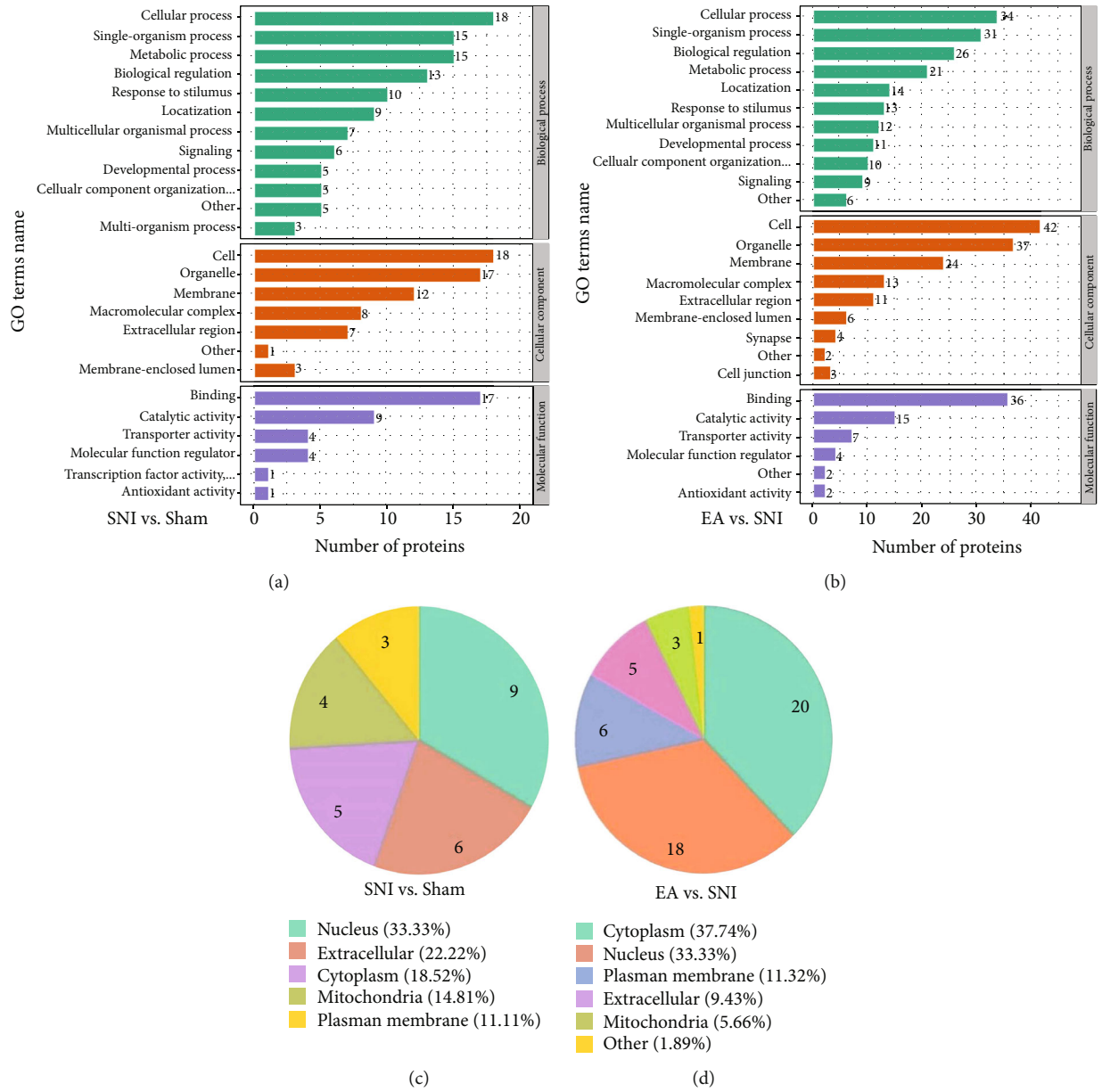

FIGURE 3: GO annotation analysis showed that the potential biomarkers identified in this study are mainly involved in SNI pathology and EA treatment: (a, b) GO annotation summarizing the numbers of SNI- and EA-regulated proteins with specific molecular function, cellular component, and biological process categories; (c, d) subcellular structural localization analysis of SNI- and EA-associated proteins.

According to the difference ratios in protein expression and the associated *P* values, we found that the most prominent upregulated or downregulated proteins, respectively, were the transmembrane protein 126A (TMEM126A) and the excitatory amino acid transporters (SLC1A2/EAAT2). Among the five known human EAAT subtypes, of the glial carriers, EAAT2 has the greatest impact on the clearance of glutamate released during neurotransmission. Spinal EAAT2 plays a critical role in both the induction and the maintenance of neuropathic pain. Sciatic nerve injury- (CCI-) induced pain caused an initial upregulation of EAAT2 on postoperative days 1–4, followed by a downregulation on days 7–14 [32]. Furthermore, upregulation and downregulation of EAAT2 are also apparent in the neuropathic pain [33]. With respect to the present study, the upregulation of hippocampal EAAT2 may be regarded as a central protective mechanism or a feedback regulation mechanism that prevents the adverse consequences of glutamate accumulation after nerve injury. Therefore, inhibition of EAAT2 expression using EA was able to alleviate the mechanical allodynia.

Further analysis of the proteomic data revealed spared nerve injury-induced downregulated proteins. These included TMEM126A, which was upregulated in the EA group, as confirmed by the ELISA/western blot results. TMEM126A encodes a mitochondrial protein found in higher eukaryotes that is important for providing mitochondrial energy. A series of studies have confirmed that auditory neuropathy is a key feature of TMEM126A-associated optic atrophy [34–

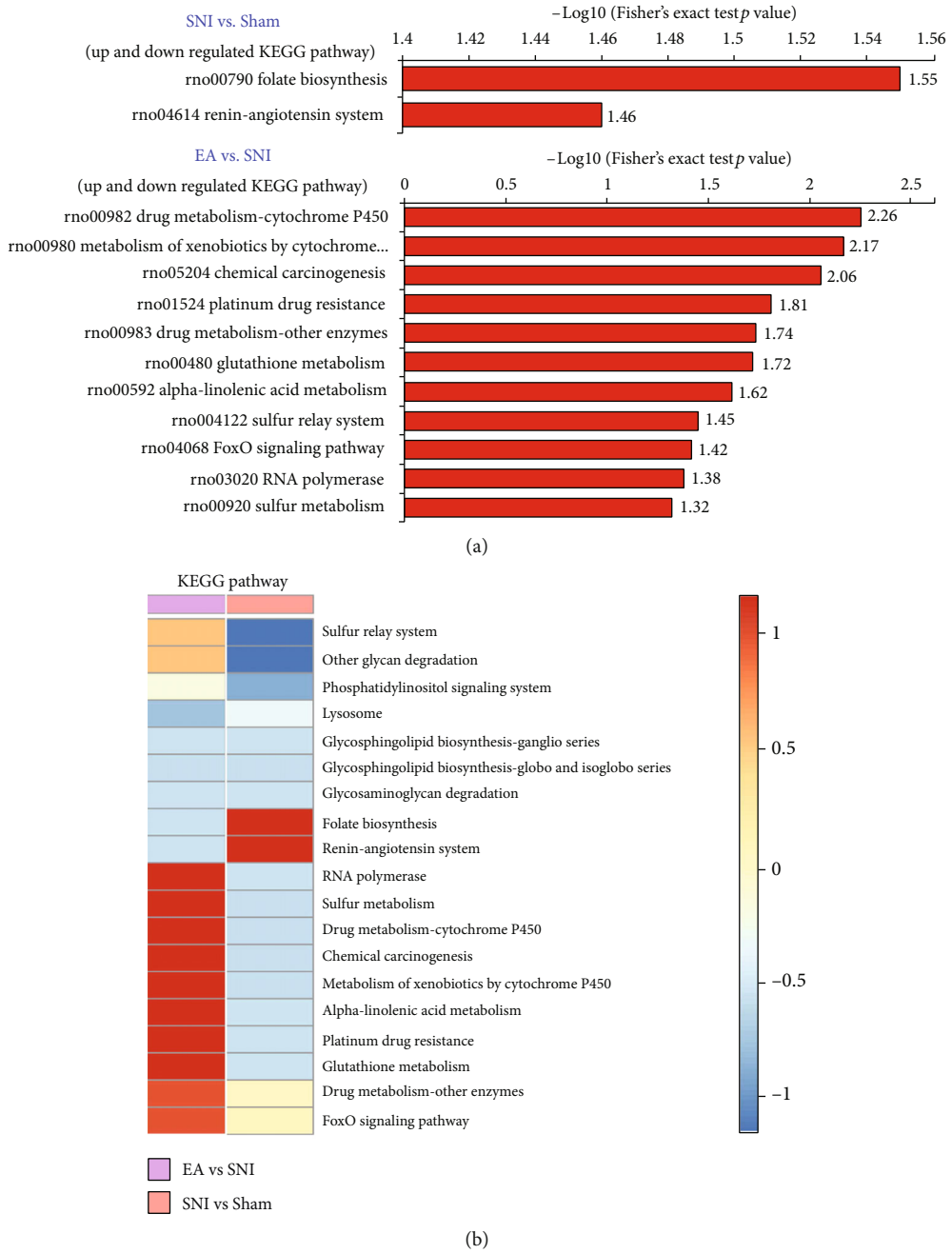

Figure 4: KEGG pathway analysis: (a) KEGG pathway enrichment analysis comparing differentially expressed proteins among the three groups; (b) KEGG pathway analysis of rats from the different groups.

37]. TMEM126A is a CD137 ligand-binding protein, which couples with the TLR4 signal transduction pathway in macrophages. It plays an important role in inflammatory immune regulation, increasing cell surface expression of proteins such as CD54, MHC II, CD40, and CD86. Cell-surface TMEM126A expression was found to increase after LPS induction in macrophages over 24 h, while CD137L expression decreased [22, 23]. Recent studies have shown that expression of the hippocampal microglia 1 surface marker CD86+ and microglial activation increase when neuropathic pain is induced by chronic peripheral nerve injury [8, 38]. In addition, expression levels of TLR4/NF-κB signaling pathway-related proteins are upregulated in the hippocampus of neuropathic pain model mice [39].

To our surprise, we found that spared nerve injury-induced neuropathic pain downregulated TMEM126 expression, while TMEM126A was upregulated after EA treatment. This study is the first to demonstrate the role of TMEM126A in the central analgesic effect of EA. Combined with the results of the studies discussed above, we speculate that EA may play an analgesic role by regulating the expression of TMEM126A. This may inhibit the expression of hippocampal microglia and the microglial M1 marker, potentially reducing hippocampal neuroinflammation. In future

experimental studies, we intend to construct a mouse model with altered expression of the TMEM126A gene or use TMEM126A shRNA to further analyze the hippocampal mechanism underlying EA-induced analgesia.

5. Conclusions

In conclusion, using the TMT labeling approach coupled with LC-MS/MS, we showed that spared nerve injury and EA stimulation drive significant changes in the levels of hippocampal proteins, especially those of inflammation-regulated proteins such as EAAT2 and TMEM126A. Our results may provide candidate protein biomarkers for the diagnosis and treatment of spared nerve injury.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

Our research was approved by the Animal Research Committee of Fujian University of Traditional Chinese Medicine (No. FJTCM2019-006).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed to the article and approved the submitted version. Degui Gong and Xiangmei Yu contributed equally to this work.

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Research Article

Comparative Transcriptome Profiling Reveals Changes of microRNAs Response to Exercise in Rats with Neuropathic Pain

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There is accumulating evidence showing that exercise therapy may play an active role in peripheral neuropathic pain (NP), but its mechanism is still unclear. Studies have found that microRNAs (miRNAs) may play a role in NP by regulating pain-related target genes. Therefore, we aimed to explore the changes of miRNA and mRNA of dorsal root ganglion (DRG) after NP in response to exercise with transcriptome technology. The chronic constriction injury (CCI) model was established, and rats were randomly allocated into three groups, namely, the sham-operated, CCI, and CCI-exercised groups. L4-L6 DRG tissue was taken for RNA-sequencing, and the differentially expressed genes (DEGs) were determined through bioinformatics analysis. Real-time PCR was used to confirm the accuracy. A total of 4 overlapping differentially expressed miRNAs and 186 overlapping differentially expressed mRNAs were identified in the two comparisons of the sham-operated group versus the CCI group and the CCI group versus the CCI-exercised group. Among these DEGs, miR-145-5p, miR-341, miR-300-5p, miR-653-5p, Atf3, Cacna2d1, Gal, and Ctss related to NP were validated by real-time PCR. DEGs between the CCI and CCI-exercised groups were enriched in HIF-1 signaling pathway, Rap1 signaling pathway, and neurotrophin signaling pathway. This study provides an understanding of the adaptive mechanisms after exercise of NP, and these DEGs in DRG might play a role in NP by stimulating the enriched pathways.

1. Introduction

Neuropathic pain (NP) is an unpleasant sensory and emotional experience caused by a lesion of or disease to the somatosensory system [1]. NP is typified by a multitude of signs, including spontaneous pain, allodynia, hyperalgesia, and paraesthesia. Unlike nociceptive pain, NP has poor response to standard analgesics; indeed, only 33%–50% patients benefit from first-line analgesics [2]. A previous report revealed that 17% of NP patients scored its impact on their quality of life as "worse than death" [3]. Thus, we must better understand NP in order to develop effective therapeutics.

Exercise has become an increasingly popular nonpharmacological approach to treat NP. Some evidence that exercise can be safe and beneficial for pain management for NP has been observed in a population of peripheral NP patients [4–7]. Aerobic exercise can increase motor conduction velocity and epidermal innervation and decrease pain ratings in people with diabetic peripheral neuropathy [8, 9]. However, although the mechanism of exercise training in improving NP induced by peripheral nerve injury has been discussed in some preclinical rodent studies, such as by decreasing pro-inflammatory cytokine expression [10–12], the molecular mechanisms triggering and maintaining these benefits remain poorly understood.

Gene expression profile research can help researchers better understand the mechanisms of NP in response to exercise. Microarray or RNA-sequencing (RNA-seq) technology has been implemented to investigate the differentially expressed genes (DEGs) in the comparison of sham-operated rats versus NP rats [13–15]. DEG analysis can help

discover quantitative changes in expression levels between experimental groups by using statistical analysis [16]. However, no study assessing the effects of exercise on dorsal root ganglion (DRG) transcriptome in NP model is yet available. In the present paper, RNA-seq was utilized to reveal the molecular responses of chronic constriction injury (CCI) rats to exercise, and DEGs and their functional associations were analyzed.

2. Methods

2.1. Animals and Groups. Adult male Sprague–Dawley rats (RRID: RGD_70508) in the weight range of 200 g to 220 g (6 weeks old) were purchased from Shanghai SLAC Laboratory. The animals were housed five to a cage with free access to food and water, and the room was maintained at a temperature of 24 ± 1°C under a 12 : 12 h dark–light cycle. All animal procedures have been approved by the Animal Care and Use Committee of Shanghai University of Sport (No. 2018006).

After 7 days of adaptive feeding, the animals were randomly allocated into three groups: rats with CCI (CCI group, $n = 6$), rats with CCI that received swimming treatment (CCI-exercised group, $n = 6$), and rats with sham operation (sham-operated group, $n = 6$). The CCI group and sham-operated group did not receive swimming treatment. Details regarding the experimental grouping and number of animals used in each part are shown in Figure 1.

2.2. Chronic Constriction Injury Models. The CCI model was constructed according to a previous description [17]. Briefly, the rats were first anaesthetized in the induction chamber with 5% isoflurane in O_2 and then administered 2% isoflurane in O_2 by using a facemask. The right hind leg of each rat was shaved and sterilized before surgery. An incision was made into the skin 3–4 mm below and parallel to the femur bone. The sciatic nerve of the right hind limb was exposed after performing blunt separation for connective tissues between the gluteus superficialis and the biceps femoris muscles. We used 4–0 chromic catgut to tie four loose ligatures around the exposed sciatic nerve at distances of 1 mm apart as described by Bennett and Xie [17]. Skin incisions were closed with 5–0 silk sutures. Sham operations involved an identical procedure to expose the sciatic nerve, but the nerve was not ligated. No additional medications or analgesics were given to the animals after surgery to reduce pain as this study observed outcomes related to pain.

2.3. Exercise Protocols. The protocol for swimming training is shown in Figure 2 and involved 6 days of habituation to swimming before surgery and 28 days of swimming training after surgery [18, 19]. Animals were placed in a plastic container (length = 82 cm, width = 60 cm, and height = 59 cm) filled with approximately 200 L of water (37°C). The training process was supervised by researchers to avoid floating behavior. Once floating behavior was observed, the researchers would nudge the nape of the rat with a pen or stir the water to create a current [20, 21]. After exercise, the rats were gently dried with a cloth towel.

2.4. Behavioral Testing. Behavioral testing was conducted before surgery and on the 3rd, 7th, 14th, 21st, and 28th postoperative day. The rats were habituated in individual glass chambers (11 cm × 22 cm × 13 cm) on a wire mesh table for 20 minutes before each test.

2.4.1. Mechanical Withdrawal Threshold (MWT). To determine the mechanical sensitivity of the hind paw, MWT was measured by using Von Frey filaments (Aesthesio, Danmic Global, USA). The test area was the midplantar surface of the hind paw, and the force of the Von Frey hairs was increased until the expected responses were induced. The range of stimulus forces was 4–180 g. Each filament from small to large was used to stimulate five times, and the force at which the rat withdrew the paw at least three times was recorded as MWT [22].

2.4.2. Thermal Withdrawal Latency (TWL). A thermal plantar algesimeter (Cat. No. 37370, Ugo Basile, Italy) was utilized to test thermal sensitivity. The rats were put on the plexiglass floor of the device with the infrared source directly beneath it. The midplantar surface of the hind paw was exposed to a radiant heat source with an intensity of 20 I.R., and a cutoff time of 30 seconds was used to protect rats from tissue injury. The latency of withdrawal was measured in seconds. This step was repeated five times at 5 min intervals between stimuli, and then, the mean TWL was calculated.

2.5. Hematoxylin-Eosin (HE) Staining. Three rats were randomly selected from each group for this analysis. The rats were deeply anesthetized with 3% pentobarbital sodium via intraperitoneal (i.p.) injection (40 mg/kg) 28 days after surgery to reduce the suffering before perfusion. In addition, the animal procedures were performed by experienced experimenters to ensure that no extra pain was caused to the rats during the experiments. The rats were perfused with 250 mL of normal saline (4°C) through the ascending aorta followed by 200 mL of 4% paraformaldehyde solution (4°C). Then, a 1 cm length of the injured sciatic nerve was obtained and postfixed in 4% paraformaldehyde solution for 90 minutes at 4°C. After postfixation, sciatic nerves were transferred to 15% sucrose solution for at least 24 hours and then transferred to 30% sucrose solution for at least 48 hours. Fixed tissues were embedded in Tissue Freezing Medium (Cat. No. 4583, SAKURA, CA, USA) and cryosectioned at 14 μm sections on a Leica CM1950 cryostat. The sections were visualized using an Olympus BX53 microscope (Tokyo, Japan) and images captured using Cell Sens Dimension software (version 1.15; Olympus; RRID:SCR_016238) with an Olympus DP80 digital camera (Olympus, Tokyo, Japan) attached to the microscope.

2.6. RNA-seq. For RNA-seq analysis, the remaining three rats were deeply anesthetized with 3% pentobarbital sodium via intraperitoneal (i.p.) injection (40 mg/kg) 28 days after surgery and perfused with 250 mL of normal saline (4°C) through the ascending aorta, and tissues of L4–L6 DRG were collected. Total RNA was extracted from the tissues using a miRNeasy Micro Kit (Cat. No. 217084, Qiagen, Hilden,

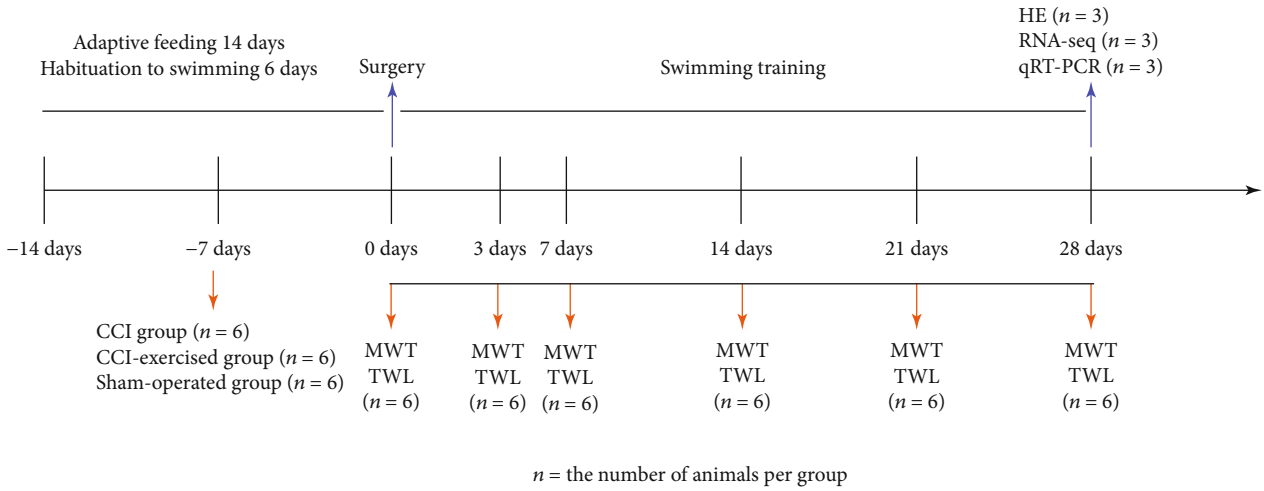


Figure 1: Experimental study design. The arrows indicate where behavioral tests were performed or samples were obtained for endpoint analysis. MWT: mechanical withdrawal threshold; TWL: thermal withdrawal latency; HE: hematoxylin-eosin staining; RNA-seq: RNA-sequencing; qRT-PCR: quantitative real-time PCR.

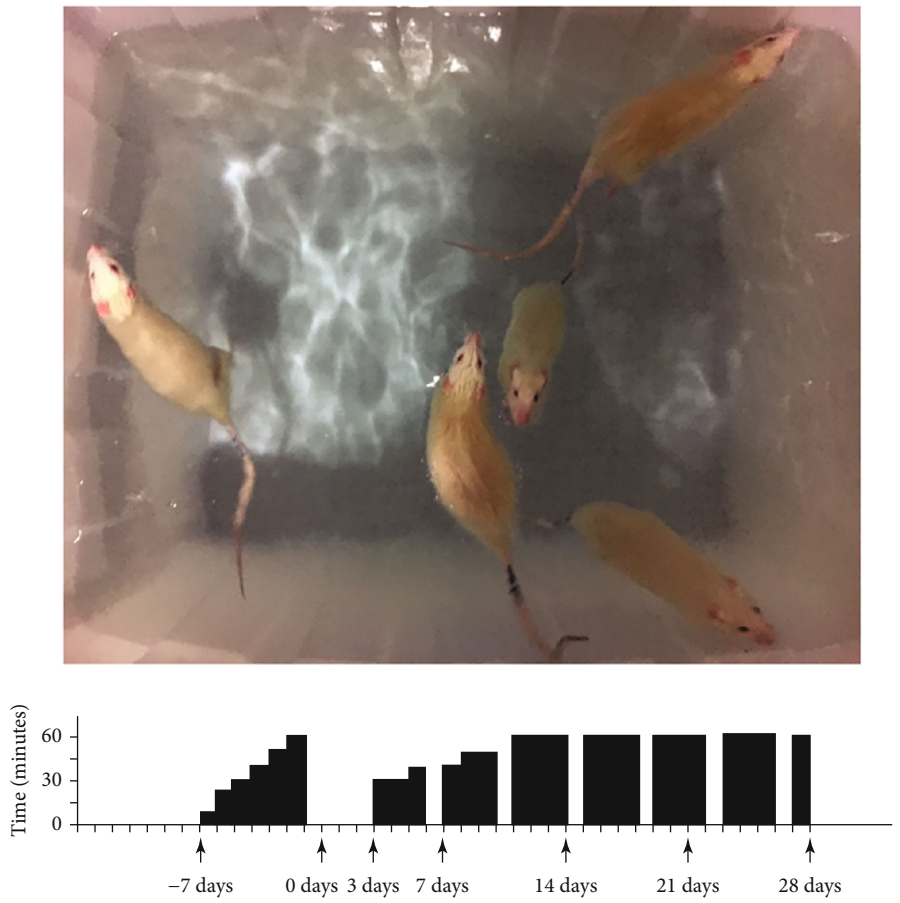


Figure 2: Protocol for swimming training. The training program involves one week of habituation to swimming before surgery and 4 weeks of formal swimming training after surgery. The black bar represents the time of training every day, and if there is no black bar, it means rest on that day.

Germany). Strict quality control of the RNA samples after RNA extraction included the following aspects: agarose gel electrophoresis to test RNA degradation and contamination, NanoPhotometer® spectrophotometry (IMPLEN, CA, USA) to assess the purity of RNA, Qubit® RNA Assay Kit (Cat. No. Q10210, Thermo Fisher Scientific, MA, USA) in Qubit® 2.0

Fluorometer (Life Technologies, CA, USA) to measure RNA concentrations, and the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Cat. No. 5067-1511, Agilent Technologies, CA, USA) to determine RNA integrity. After the RNA samples met the quality requirements, the NEBNext® Multiplex Small RNA Library Prep Set for Illumina® (Cat. No. E7300L, NEB, MA, USA) and NEBNext® UltraTM RNA Library Prep Kit for Illumina® (Cat. No. E7530L, NEB, MA, USA) were utilized to create sequencing libraries. Finally, the library preparation was sequenced on the Illumina Hiseq 2500/2000 platform.

2.7. Bioinformatics Analysis. First, the quality of raw data obtained from RNA-seq was evaluated, including filtering the raw data, checking the error rate, and GC content distribution. Then after mapping, assembly was done followed by quantification. To analyze the RNA-seq data, the DESeq2 R package (RRID: SCR_015687) was utilized to calculate DEGs in the two comparisons (sham-operated group versus CCI group and CCI group versus CCI-exercised group) and request the p values less than 0.05. The miRNA target genes were predicted as the intersection of the two software, miRanda and RNAhybrid. We then cross the predicted miRNA target genes with the mRNA-seq results to obtain their intersection genes. Gene Ontology (GO; RRID: SCR_002143) enrichment analysis was used on the DEGs by GOseq software (RRID: SCR_017052). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (RRID: SCR_012773) was implemented to understand the functions and utility of the biological system identified (http://www.genome.jp/kegg/) [23], and KEGG Orthology-Based Annotation System software (RRID: SCR_006350) was utilized to identify the enriched pathways [24]. p values less than 0.05 were set as enrichened significance.

2.8. Quantitative Real-Time PCR (qRT-PCR) Analysis. The DEGs were validated through qRT-PCR of the nine sequenced samples to identify the accuracy of the RNA-seq results. The first-strand cDNA was synthesized by using the miScript II RT Kit (Cat. No. 218161, Qiagen, Hilden, Germany). Then, the first-strand cDNA was amplified by using the miScript® SYBR® Green PCR Kit (Cat. No. 218073, Qiagen, Hilden, Germany) and QuantiNova SYBR Green PCR Kit (Cat. No. 208054, Qiagen, Hilden, Germany) with a real-time PCR system (Applied Biosystems StepOnePlus, CA, USA). The primers used are provided in Table 1. The comparative Ct method ($\Delta\Delta$Ct) was used to quantify the expression of these miRNAs and mRNAs, and relative expressions were normalized to that of U6 and 18S RNA by using the $2^{-\Delta\Delta Ct}$ method.

2.9. Statistical Analysis. All statistical calculations were performed using SPSS software (version 16.0, SPSS, Inc., Chicago, IL, USA; RRID: SCR_002865). Data of the characteristics of each group are expressed as the mean ± standard error of mean (SEM). Results from the behavioral tests were analyzed using two-way repeated-measure analysis of variance (ANOVA). In addition, results of qRT-PCR were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. p values less than 0.05 were set as statistical significance.

Table 1: The primers used in qRT-PCR.

Gene	5′ to 3′
U6 forward	CGCAAGGATGACACGCAAATTCG
mir-145-5p forward	CAGTTTTCCCAGGAATCCCT
miR-653-5p forward	TTGAAACATTCTCTACTGAAC
miR-341 forward	GTCGATCGGTCGGTCGGT
miR-300-5p forward	TTGAAGAGAGGTTATCCTTTGT
Universal miRNA/U6 reverse primer	Provided by the reagent kit
Atf3 forward	GCCATCCAGAACAAGCACC
Atf3 reverse	CACTTGGCAGCAGCAATTT
Gal forward	GGTTCCCACCACTGCTCAAG
Gal reverse	CCTTTGTTGGCATCCCGAG
Cacna2d1 forward	GGCGTTGATGTGTCTCTGGA
Cacna2d1 reverse	AGTGTGACCGGCTCCTTTG
Ctss forward	TGGTGGATTGCTCAACCGAA
Ctss reverse	TCAGAGCTTCTTCATCGCCG
18 s rRNA forward	GCTTAATTTGACTCAACACGGGA
18 s rRNA reverse	AGCTATCAATCTGTCAATCCTGTC

3. Results

3.1. Animal Characteristics. The characteristics of the three groups are illustrated in Figure 3. Body weight, MWT, and TWL were measured 0, 3, 7, 14, and 28 days after surgery. The CCI and CCI-exercised groups showed no significant difference in body weight when compared with the sham group (Figure 3(a)). Results also demonstrated that the MWTs of the CCI and CCI-exercised groups were significantly lower than that of the sham-operated group on the 3rd ($p < 0.01$), 7th ($p < 0.01$), and 14th ($p < 0.01$) postoperative days (Figure 3(b)). On the 21st ($p < 0.01$) and 28th ($p < 0.01$) postoperative days, the level of MWT in the CCI-exercised group was significantly increased compared with that in the CCI group. Furthermore, no significant difference was observed between rats in the sham and CCI-exercised groups on the 21st and 28th postoperative days. In contrast to exercised rats, MWT was significantly reduced in CCI rats ($p < 0.01$). The results presented in Figure 3(c) show that the TWL, CCI, and CCI-exercised groups indicated reduced thermal hypersensitivity on postoperative days 3 ($p < 0.01$), 7 ($p < 0.01$), and 14 ($p < 0.01$) compared with the sham-operated group. Similarly, swimming increased TWL in the CCI-exercised group on days 14 ($p < 0.01$), 21 ($p < 0.01$), and 28 ($p < 0.01$) compared with that of the CCI group.

3.2. Histopathology Analysis of Sciatic Nerves. HE staining was used to observe the histopathological changes of the sciatic nerve in each group, further confirming whether the establishment of the CCI model was successful. As shown in Figure 4, the HE sections showed that sciatic nerve fibers were scattered, myelin sheath vacuolated, Schwann cells

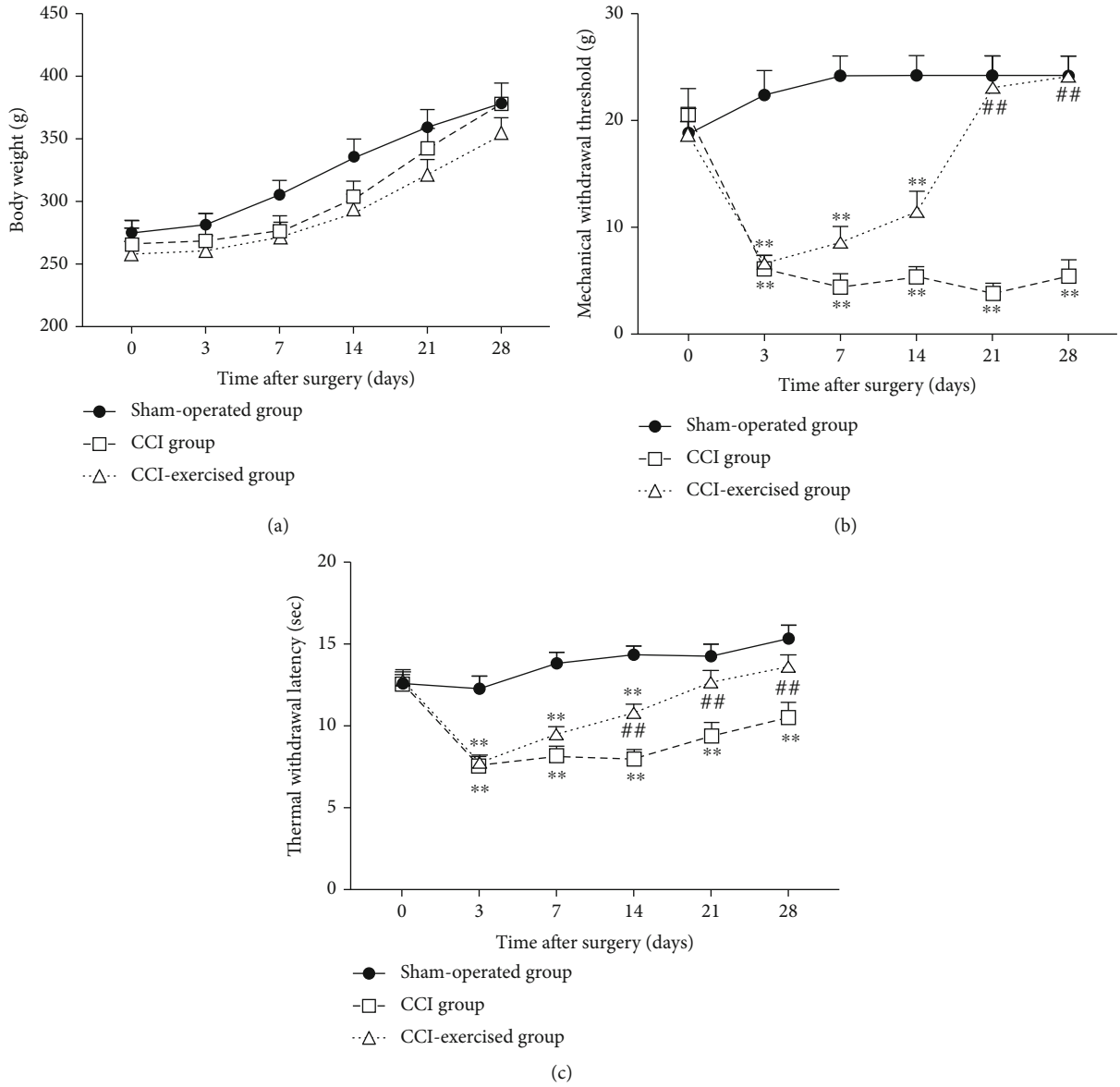


Figure 3: Body weight and nociceptive behavior in different time courses. (a) Time courses of body weight, (b) mechanical withdrawal threshold, and (c) thermal withdrawal latency. Analyses of data were done by two-way ANOVA for repeated measures. Values indicate the mean ± standard error of mean ($n = 6$ animals/group). $^{*}p < 0.05$ and $^{**}p < 0.01$, for comparisons of the sham-operated group vs. the chronic constrictive injury (CCI) group or CCI-exercised group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ for comparisons of the CCI-exercised group vs. the CCI group.

proliferated, and peripheral inflammatory cells infiltrated in the CCI group, which indicated the success of the CCI model. The morphology of nerve fibers, proliferation of Schwann cells, and infiltration of peripheral inflammatory cells in CCI exercise group were significantly improved, which was similar to that in the sham-operated group.

3.3. Identification of Differentially Expressed Genes. The optical density 260/280 of all RNA samples ranged from 1.9 to 2.2, and the number of RNA integrity was greater than 7. The quality of raw data was assessed, and the results are shown in Tables S1–S2. Figure 5 shows the results of differentially expressed miRNAs in the two comparisons (sham-operated group versus CCI group and CCI group versus CCI-exercised group). Among the identified differentially expressed miRNAs, 67 genes were found to be differentially expressed between the sham-operated and CCI groups, with 21 upregulated and 46 downregulated miRNAs (Figure 5(a)). After 4 weeks of swimming, 7 miRNAs with significantly changed expression were observed in CCI-exercised rats compared with CCI rats. Among these miRNAs, 5 and 2 miRNAs were upregulated and downregulated (Figure 5(b)), respectively. Four overlapping differentially expressed miRNAs (miR-145-5p, miR-341, miR-300-5p, and miR-653-5p) were identified in a Venn diagram (Figure 5(c)). Hierarchical clustering analysis of

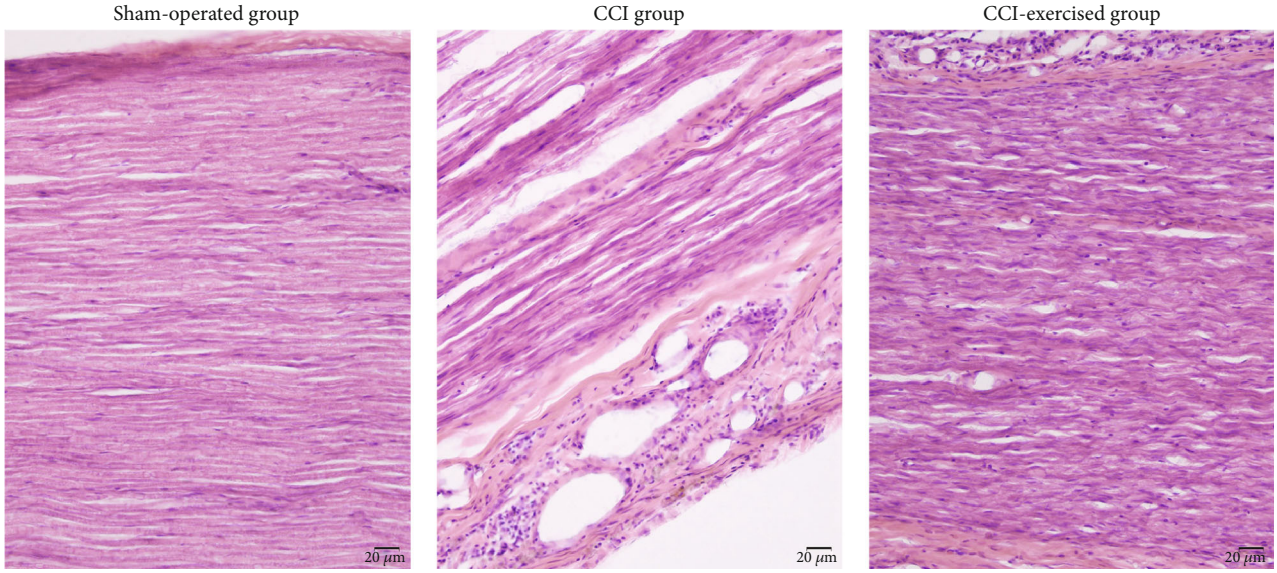


Figure 4: Histological changes of sciatic nerves. The hematoxylin-eosin (HE) sections were observed under light microscopy (×200, bar = 20 μm).

differentially expressed miRNAs was used to determine the clustering pattern of the three groups (Figure 5(d)).

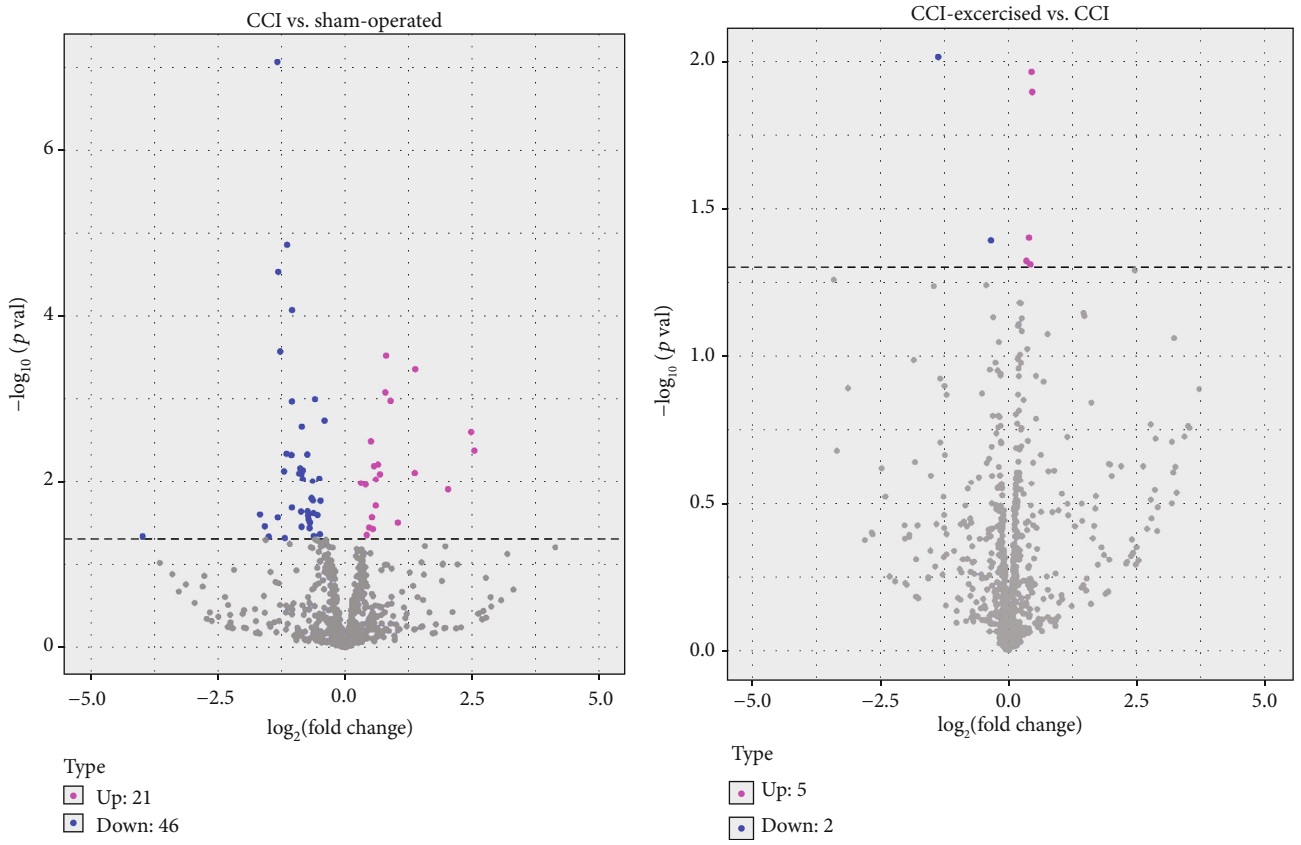
In addition, Figure 6 shows the results of differentially expressed mRNAs. The volcano plot in Figure 6(a) indicates that 363 and 244 mRNAs are upregulated and downregulated, respectively, between the CCI and sham groups. After 4 weeks of swimming, 169 and 242 mRNAs were upregulated and downregulated, respectively, as observed in Figure 6(b). A total of 186 overlapping differentially expressed mRNAs were found in Figure 6(c). Among 186 differentially expressed mRNAs, 14 mRNAs were identified to be related to NP and multiple-related phenotype by searching the Rat Genome Database (RGD, Table 2). According to the disease annotations in RGD, 6, 4, 2, 6, and 6 mRNAs were associated with nervous system disease, neuralgia, pain, hyperalgesia, and inflammation, respectively. Hierarchical clustering analysis of differentially expressed mRNA was used to show the clustering pattern of the differential mRNA expression of the three groups (Figure 6(d)).

Two softwares are used to predict target genes in overlapping differentially expressed miRNAs (miR-145-5p, miR-341, miR-300-5p, and miR-653-5p), and the result is that a total of 125 target genes can be predicted by the four miRNAs. Then, it was crossed with the sequencing results of 186 overlapping differentially expressed mRNAs to obtain a total of 1 cross gene (Lrguk).

3.4. GO Analysis of DEGs. According to the target gene candidates of differentially expressed miRNAs, GO analysis was performed to explore the biological process (BP), cellular component (CC), and molecular function (MF) of DEGs in the two comparisons (sham-operated group versus CCI group and CCI group versus CCI-exercised group). Figure 7(a) shows the top 10 GO terms in the comparison between the sham-operated and CCI groups ($p < 0.05$). The most significantly enriched BPs were intracellular signal transduction, positive regulation of cellular process, and cellular component organization. The most significantly enriched CCs were cytoplasm, intracellular part, and intracellular. The most significantly enriched MFs were protein binding, MF regulator, and SH3 domain binding. The top 10 GO terms in the comparison between the CCI and CCI-exercised groups are shown in Figure 7(b). The most significantly enriched BPs were regulation of cellular respiration, positive regulation of kinase activity, and regulation of vascular wound healing. The most significantly enriched CCs were apical cytoplasm, actomyosin contractile ring, and postsynaptic endocytic zone. The most significantly enriched MFs were kinase activator activity, protein kinase activator activity, and p53 binding.

The enriched GO terms of differentially expressed mRNAs between the two comparisons are shown in Figures 7(c) and 7(d) ($p < 0.05$). Between the sham-operated and CCI groups, the most significantly enriched BPs were defense response to bacterium, response to bacterium, and response to another organism. The most enriched CCs were ERMES complex, ER–mitochondrion membrane contact site, and signal recognition particle receptor complex. The most significantly enriched MFs were gastrin receptor activity, cholecystokinin receptor binding, and type B gastrin/cholecystokinin receptor binding (Figure 7(c)). Between the CCI and CCI-exercised groups, the most significantly enriched BPs were defense response to bacterium, defense response, and response to bacterium. The most enriched CCs involved CatSper, dystroglycan, and sarcoglycan complexes. The most significantly enriched MFs were natural killer cell lectin-like receptor binding, methyltransferase activity, and transferase activity (Figure 7(d)).

3.5. KEGG Pathway Analysis of DEGs. The target gene candidates of differentially expressed miRNAs and differentially expressed mRNAs in the two comparisons (sham-operated



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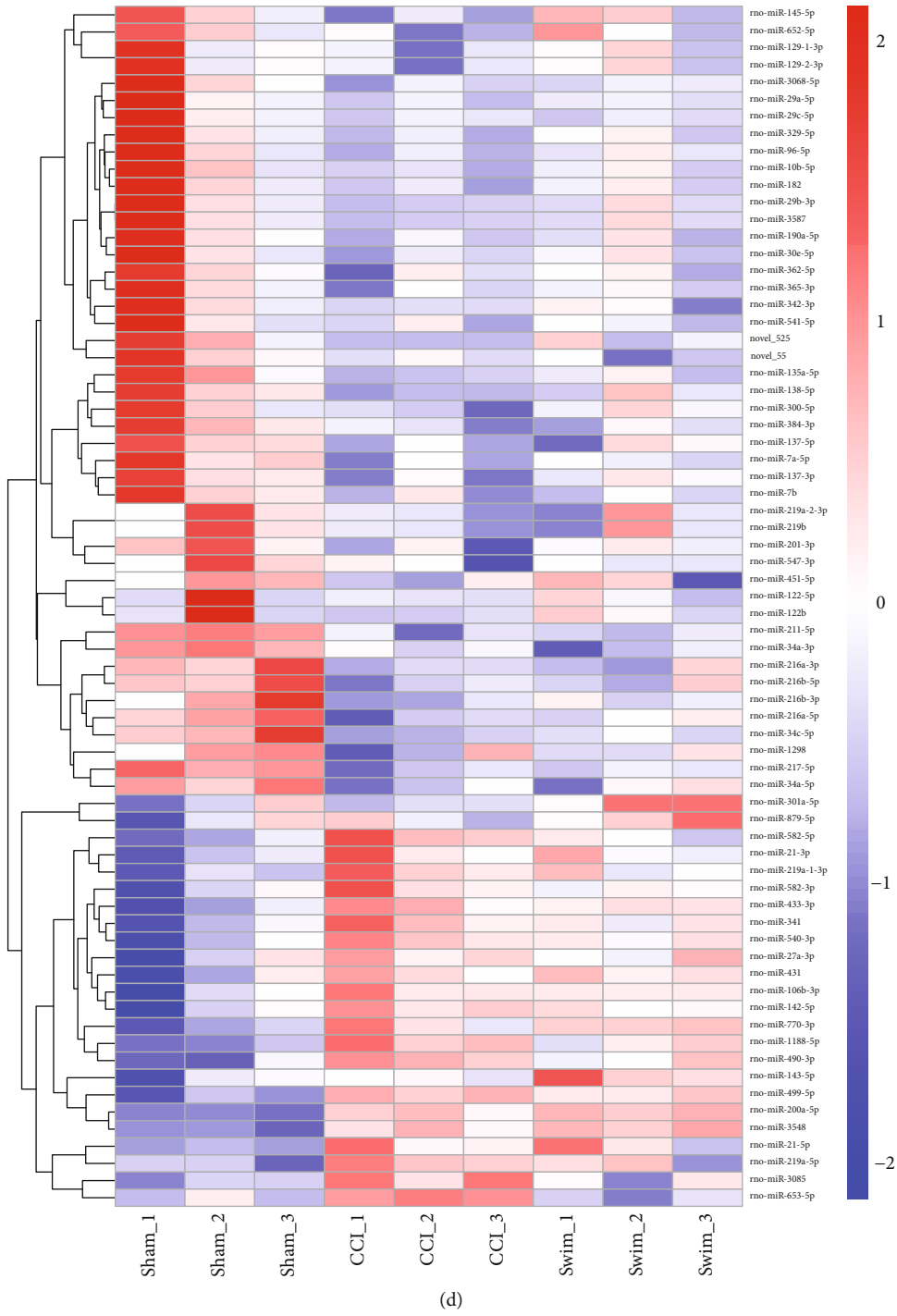
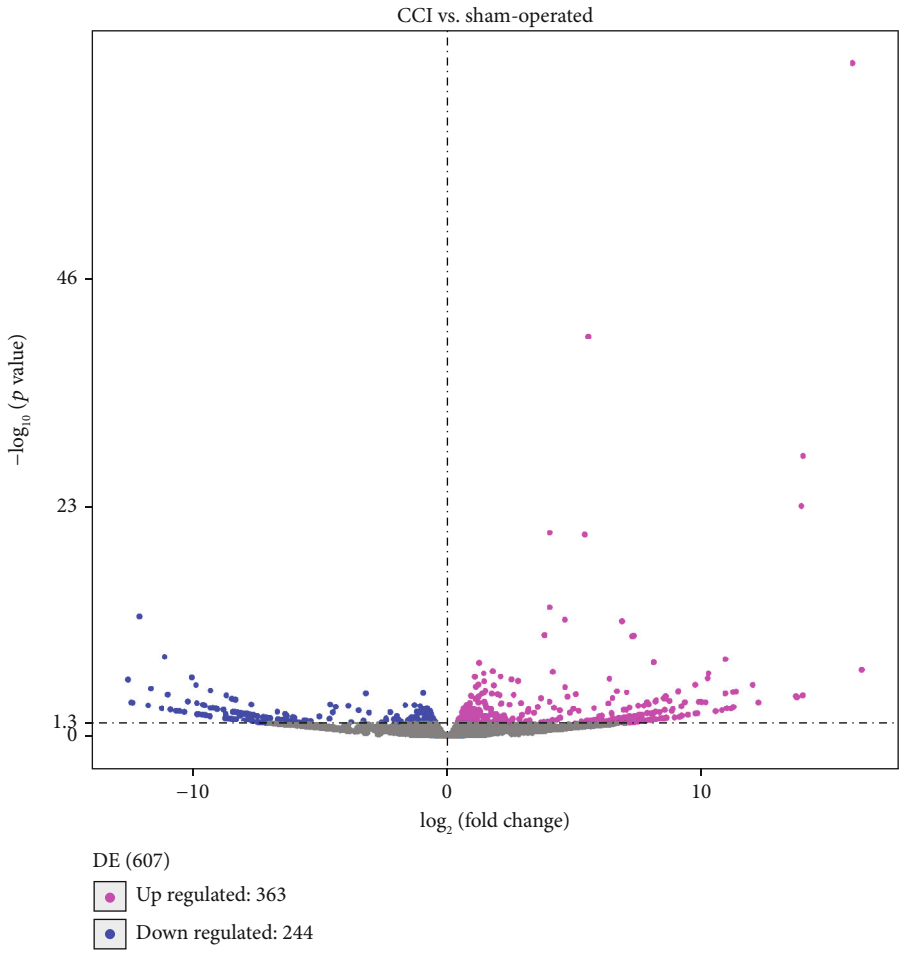


(d)

Figure 5: Expression profile changes in microRNAs (miRNAs) in the dorsal root ganglion (DRG). (a) Volcano plots of RNA-sequencing data showing differentially expressed miRNAs between the chronic constrictive injury (CCI) and sham-operated groups. (b) Volcano plot showing differentially expressed miRNAs between the CCI and CCI-exercised groups. Magenta dots represent genes with significantly upregulated expression, blue dots represent genes with significantly downregulated expression, and grey dots represent genes with no significant difference. (c) Overlap of differentially expressed miRNAs between the two comparisons shown as a Venn diagram. (d) Heatmap showing the relative expression levels of differentially expressed miRNAs among the three groups. Upregulated and downregulated genes are colored red and blue, respectively ($n = 3$ animals per group).

group versus CCI group and CCI group versus CCI-exercised group) were subjected to KEGG pathway analysis (Figures 8(a)–8(d)). For miRNAs, 20 pathways were enriched in both comparisons, and the top 5 significantly enriched KEGG pathways included natural killer cell-mediated cytotoxicity, HTLV-I infection, bacterial invasion



(a)

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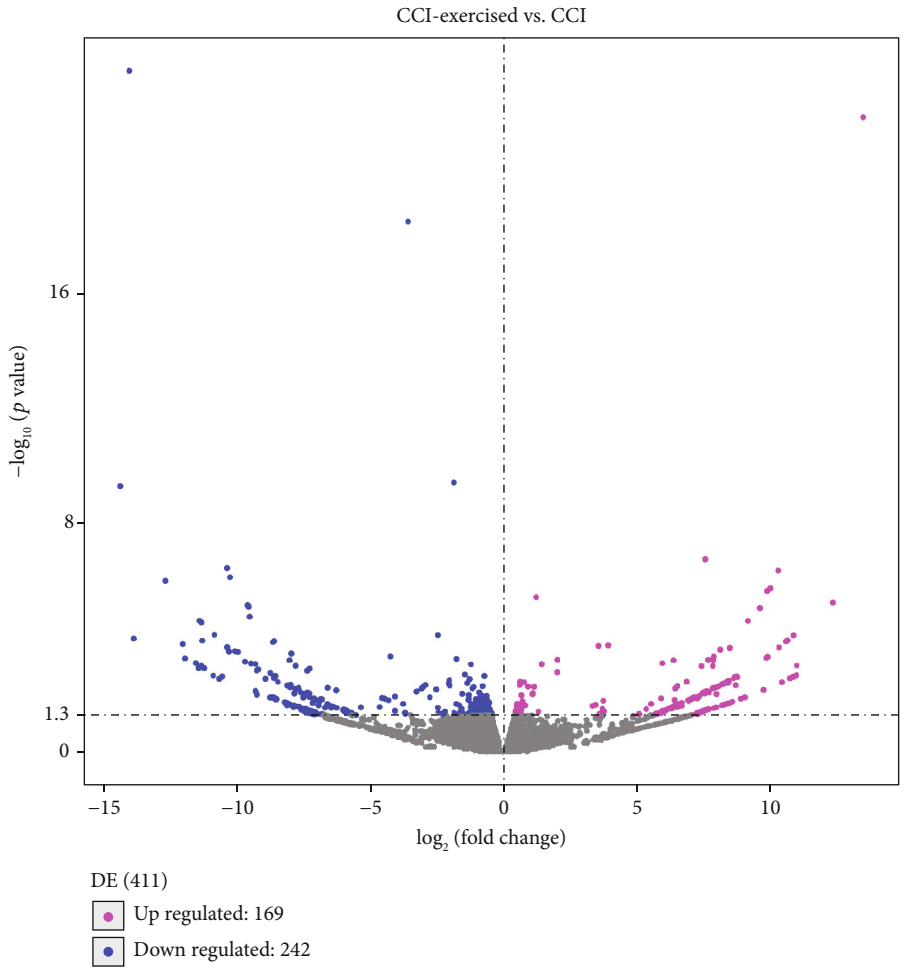

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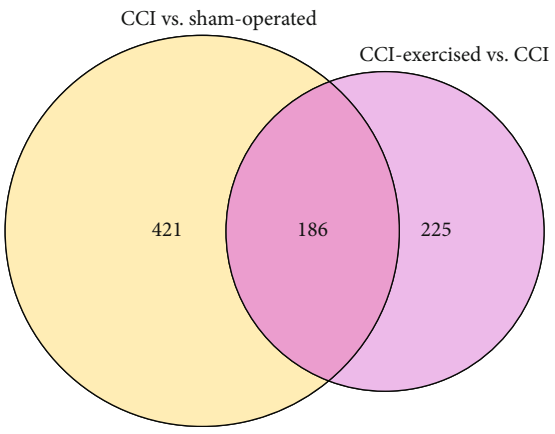

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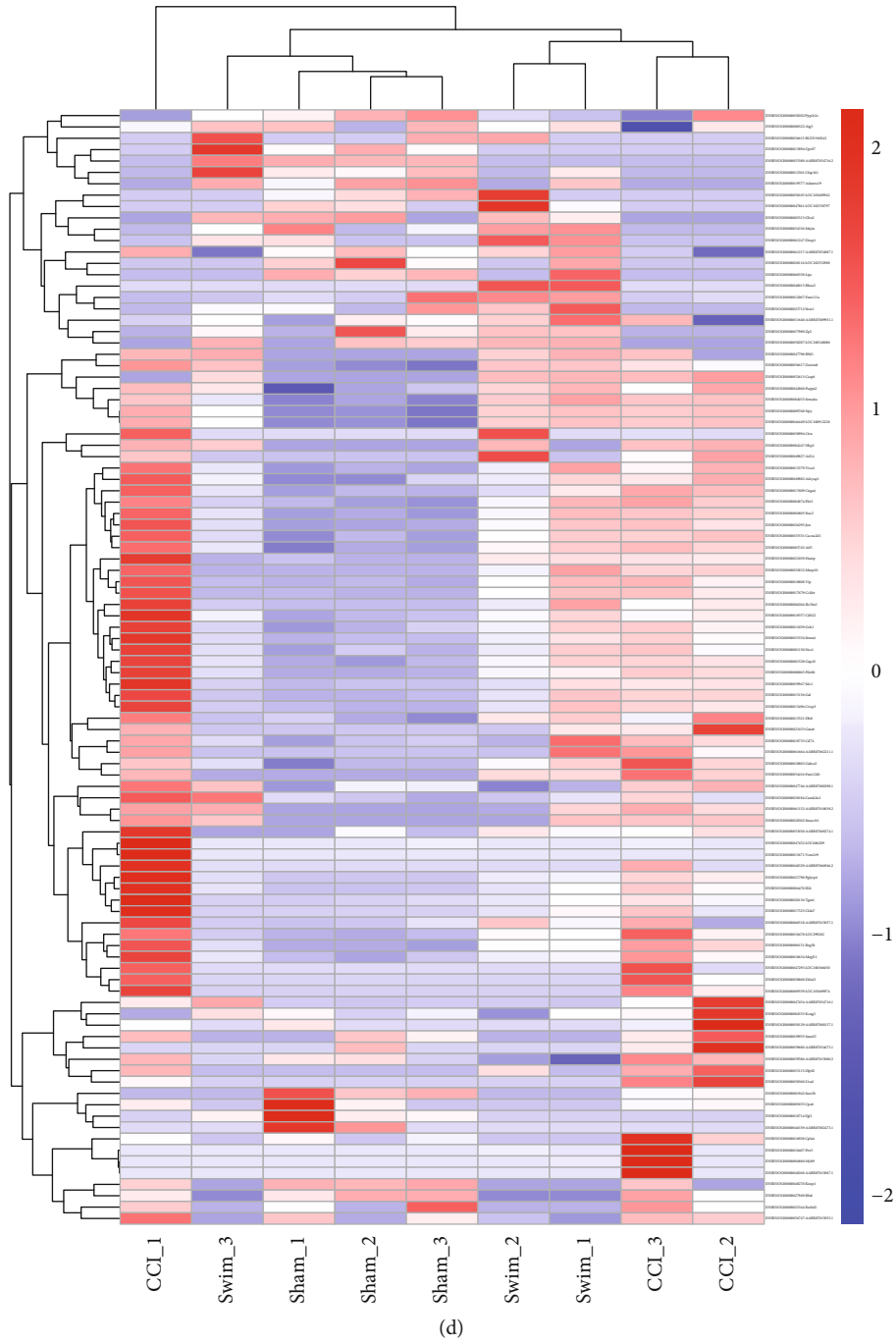


(d)

Figure 6: Expression profile changes in mRNA in the dorsal root ganglion (DRG). (a) Volcano plot indicating upregulated and downregulated differentially expressed mRNAs between the chronic constrictive injury (CCI) and sham-operated groups. (b) Volcano plot indicating upregulated and downregulated differentially expressed mRNAs between the CCI and CCI-exercised groups. Magenta dots represent genes with significantly upregulated expression, blue dots represent genes with significantly downregulated expression, and grey dots represent genes with no significant difference. (c) Overlap of differentially expressed mRNAs between the two comparisons shown as a Venn diagram. (d) Heatmap showing the relative expression levels of differentially expressed mRNAs among the three groups. Upregulated and downregulated genes are colored red and blue, respectively ($n = 3$ animals per group).

of epithelial cells, cell adhesion molecules, and Fc gamma R-mediated phagocytosis ($p < 0.05$). Differentially expressed mRNAs were also analyzed, and four signaling pathways were significantly enriched between the CCI and sham-operated groups, including folate biosynthesis, cAMP signaling pathway, tight junction, and malaria ($p < 0.05$). Four pathways were significantly enriched between the CCI and CCI-exercised groups, including HIF-1 signaling pathway, purine metabolism, protein export, and regulation of autophagy ($p < 0.05$).

Table 2: Comparison of differentially expressed mRNAs with NP-related phenotypes in RGD database.

Gene	Comparison1 p value	Comparison1 $\log_2$ FC	Comparison2 p value	Comparison2 $\log_2$ FC	NSD	Neuralgia	Pain	Hyperalgesia	Inflammation
Atf3	$3.20E-21$	4.045819	0.001948	-1.442530	√				
Gal	$7.18E-11$	3.829123	0.003094	-2.022188		√		√	√
Cacna2d1	$4.61E-08$	1.261635	0.014522	-0.607313		√	√	√	
Nos1	$3.00E-07$	1.799434	0.030345	-0.835820	√	√		√	
Reg3b	$3.49E-07$	4.162273	0.004735	-2.012063	√				
Adcyap1	$6.55E-06$	1.235189	0.019065	-0.639252				√	
Gch1	$6.40E-05$	1.984458	0.047527	-0.959824			√		
Sdc1	$7.90E-05$	2.078746	0.013891	-1.300921					√
Il1a	0.000680	7.930640	0.000627	-8.012905				√	√
Tnfaip6	0.000696	2.455342	0.003988	-2.044203					√
Ctss	0.004446	0.836964	0.017493	-0.774995	√	√		√	
Ltf	0.010002	7.393181	0.009157	-7.477871					√
Spp1	0.010235	0.605711	0.036589	-0.494265	√				√
Cyp11a1	0.026784	-7.823132	0.004030	8.228974	√				

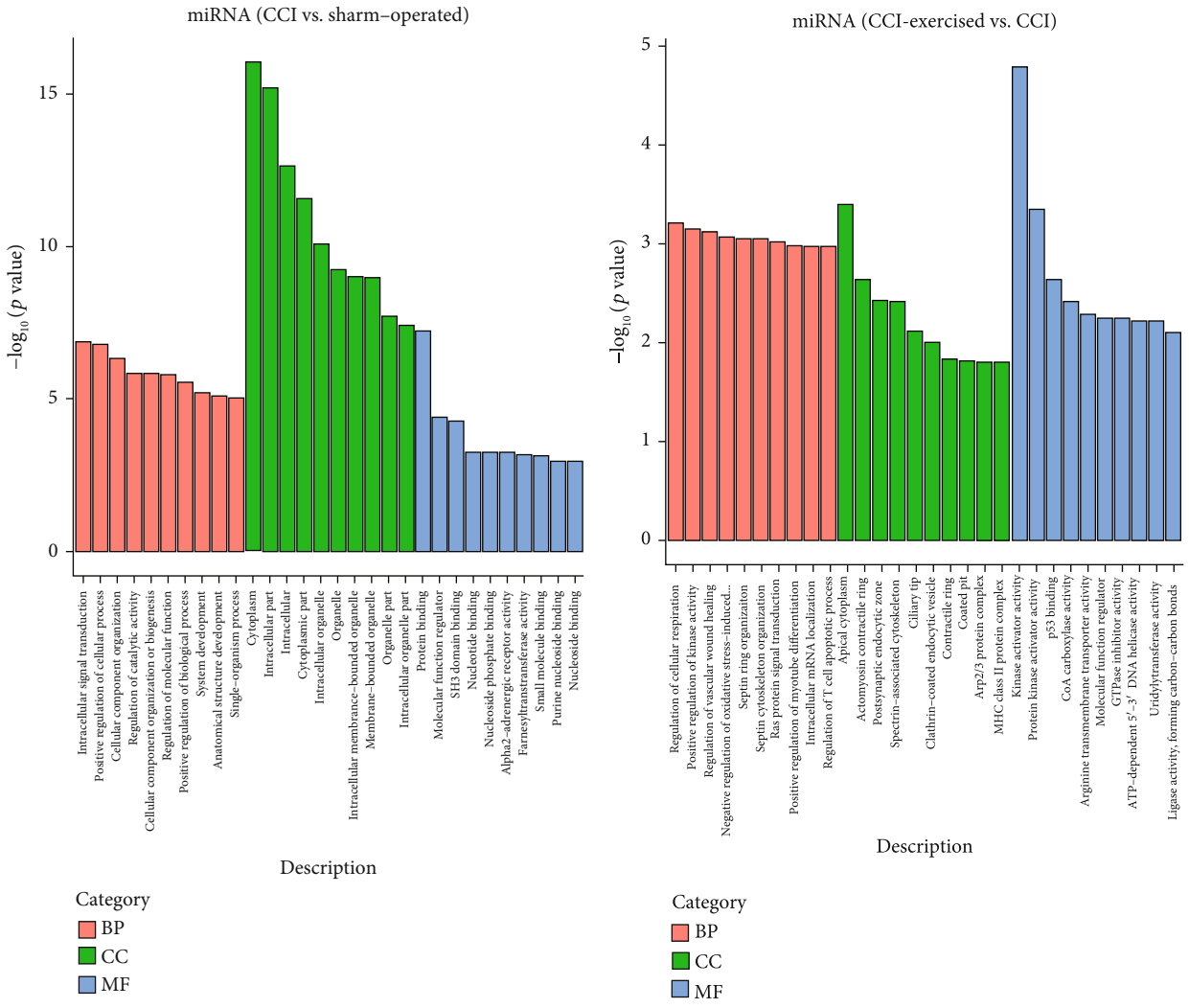
Comparison1 represents the sham-operated group vs. CCI group. Comparison2 represents the CCI group vs. CC-exercised group; FC: fold change; NDS: nervous system disease.

3.6. qRT-PCR Validation of RNA-seq Data. Four differentially expressed miRNAs (miR-145-5p, miR-341, miR-300-5p, and miR-653-5p) and four differentially expressed mRNAs (Atf3, Cacna2d1, Gal, and Ctss) were analyzed by qRT-PCR (Figures 9(a)–9(h)) to confirm the accuracy of the RNA-seq results. All qRT-PCR results were consistent with results obtained from the RNA-seq data.

4. Discussion

In this study, the transcriptome responses of NP rats to exercise in the DRG tissue were examined to explore adaptive mechanisms after exercise. Specifically, differentially expressed miRNAs and mRNAs in the L4–L6 DRG were determined by comparisons between CCI, sham-operated, and CCI-exercised rats, after which the functional associations of these DEGs were analyzed. DRG neurons link the peripheral nervous system to the central nervous system, which regulates sensory pathways by receiving nociceptive afferents (e.g., heat, cold, pressure, and chemicals) and then transmitting the information to the spinal dorsal horn [25]. We chose the CCI model, which has been used as a classic model of NP induced by peripheral nerve injury, to simulate the symptoms of chronic sciatic nerve compression in humans [26, 27]. The gene location of miRNA is highly conserved in different species and shows a high degree of homology in sequence [28, 29]. Moreover, the expression patterns of homologous miRNAs have been found to be comparable between organs in human and rat. Here, we want to investigate the miRNAome changes in NP using rats as the animal model and analyze biological functions and pathways of DEGs [30]. Among the differentially expressed miRNAs observed, the expressions of miR-145-5p and miR-300-5p are negatively affected by CCI and positively recovered by exercise. By contrast, miR-653-5p and miR-341 increased after CCI, and these changes could be returned to the control level by exercise. This result suggests that these differentially expressed miRNAs in the DRG may be potential therapeutic goals. Our transcriptome data are consistent with the results of previous studies. For example, Pang et al. studied spinal cord tissue and demonstrated that the expression of miR-145-5p on the 1st, 3rd, 5th, and 7th postoperative days of CCI is significantly decreased compared that observed 1 day before surgery [31]. The genome-wide profile of miRNAs determined in a clinical study indicated that miR-145-5p expression is significantly lower in patients with chronic pain compared with healthy persons [32]. Another study reported that in CCI animal models, overexpression of miR-145-5p was found to significantly improve hyperalgesia induced by mechanical and thermal stimuli [33]. This suggests that miR-145-5p could be involved in exercise to improve the mechanism of CCI-induced hyperalgesia in rats. Using microarray analysis and qRT-PCR analysis, Li et al. revealed that miR-341 expression is significantly upregulated in the DRG of rats with NP compared with that of rats in the normal and sham-operated groups [14].

According to the differentially expressed mRNAs in our transcriptome data, 186 genes in the DRG show altered expression in the both two comparisons of the sham-operated group versus the CCI group and the CCI group versus the CCI-exercised group. Among these differentially expressed mRNAs, according to RGD and previous research, the expressions of Atf3, Ctss, Cacna2d1, and Gal may correlate with NP and contribute to allodynia and hyperalgesia in NP rats. Atf3 is a member of the ATF/cyclic AMP response element-binding transcription factor family and known to be a neuronal injury marker. Previous studies have showed



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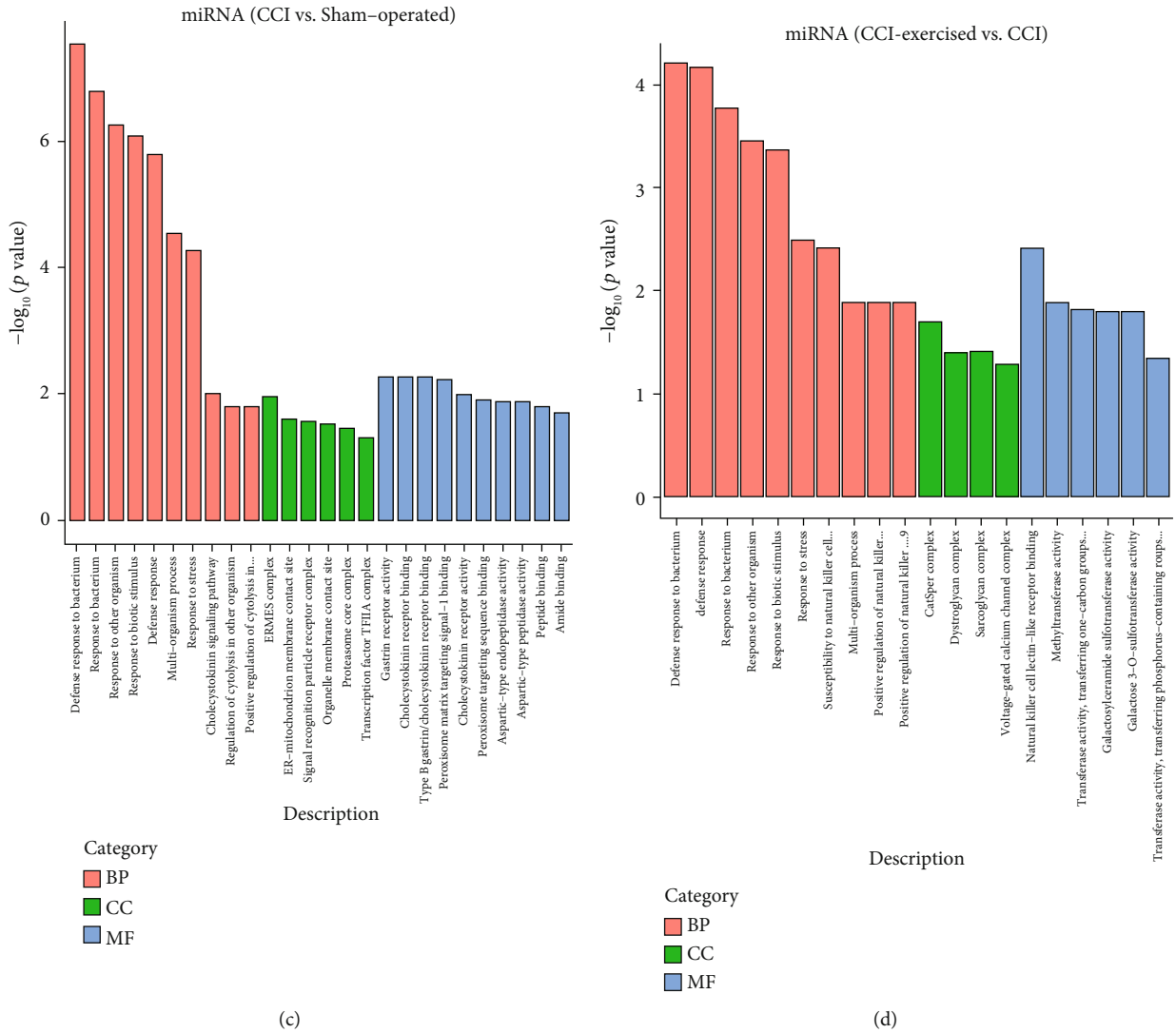
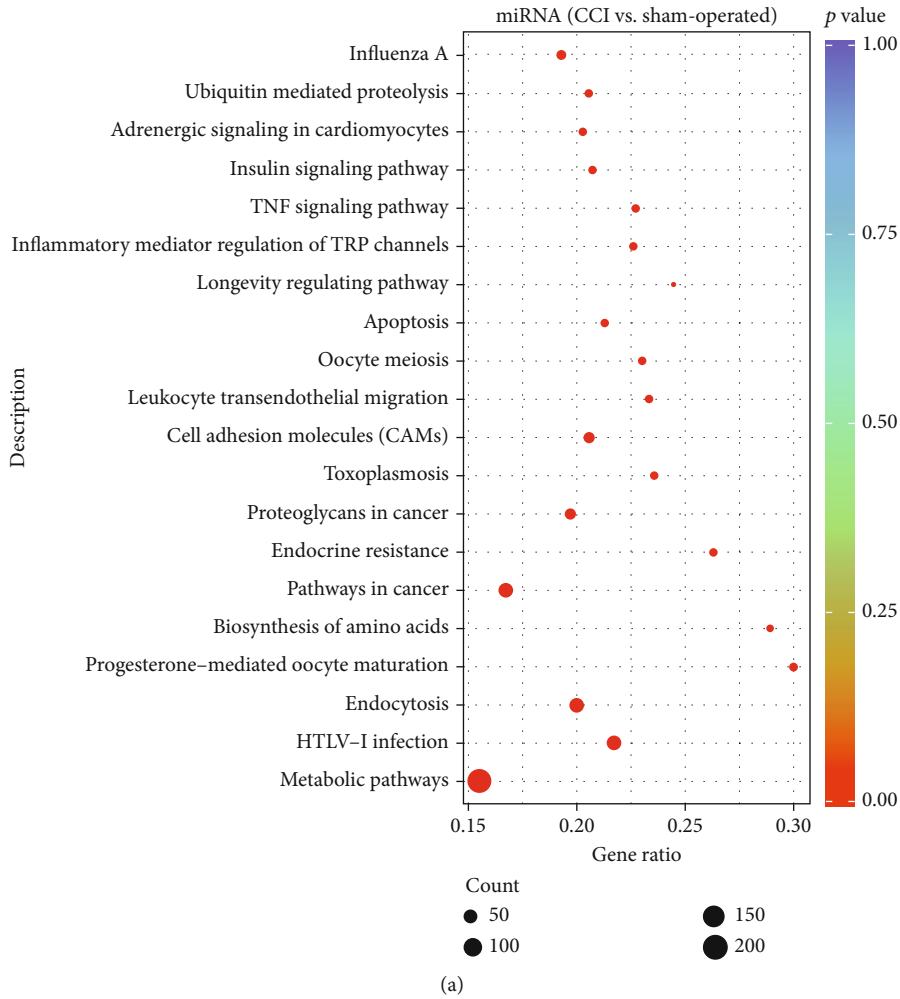


Figure 7: Gene Ontology (GO) analysis of differentially expressed genes (DRGs). The top 10 high-enrichment score terms of differentially expressed microRNAs (miRNAs) are shown in the histography (a) between the sham-operated group and the chronic constrictive injury (CCI) group and (b) between the CCI group and the CCI-exercised group. The top 10 high-enrichment score terms of differentially expressed mRNAs are shown in the histography (c) between the sham-operated group and the CCI group and (d) between the CCI group and the CCI-exercised group ($n = 3$ animals per group).

that Atf3 is upregulated in DRG neurons after periphery nerve injury, including CCI, diabetic peripheral neuropathy, and L5 spinal nerve ligation. Moreover, these studies also showed that 4 weeks of exercise training could restore the level of Atf3. [34–37]. Another gene Ctss plays a role in the maintenance of NP. A previous study described that the mRNA expression of lysosomal cysteine protease Ctss in the DRG increased after NP induced by peripheral nerve injury, and inhibitors of Ctss could reverse mechanical allodynia and hyperalgesia [38]. This finding is consistent with our transcriptome data of the comparison between the sham-operated and CCI groups. Ctss has been found to be expressed in microglia in the spinal cord and brain after NP [39]. Thus, the elevated expression of Ctss in the peripheral and central nervous systems may contribute to NP of CCI rats. Cacna2d1 is expressed in neurons throughout the central nervous system, with enrichment in the DRG, spinal dorsal horn, and cortical and hippocampal neurons [40]. Previous studies reported that nerve injury increases the expression of Cacna2d1 [21, 41–43]. Furthermore, Cacna2d1 is a voltage-gated calcium channel subunit, and its dysregulation may contribute to NP states by modulating synaptic transmission and plasticity, such as abnormal synaptogenesis [44–46]. Such changes can alter the activities of neuronal networks that fundamentally contribute to the cellular basis of NP development [47]. The Gal neuropeptide shows neuroprotection against the damage induced by nerve injury. Xu et al. [48] found that the expression levels of Gal and its receptors GalR1 and GalR2 were significantly increased in both DRG and spinal dorsal horn of bilateral CCI rats and decreased after intrathecal injection of exogenous Gal. This suggests that Gal may participate in reducing NP by



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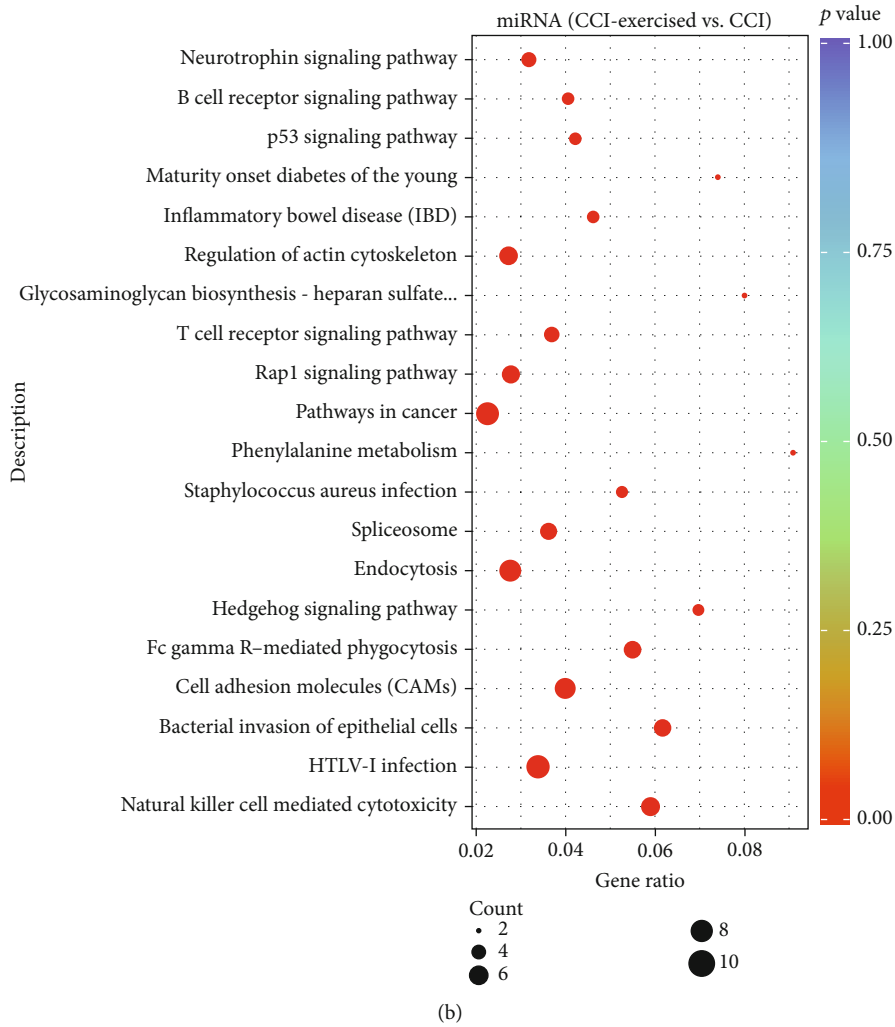

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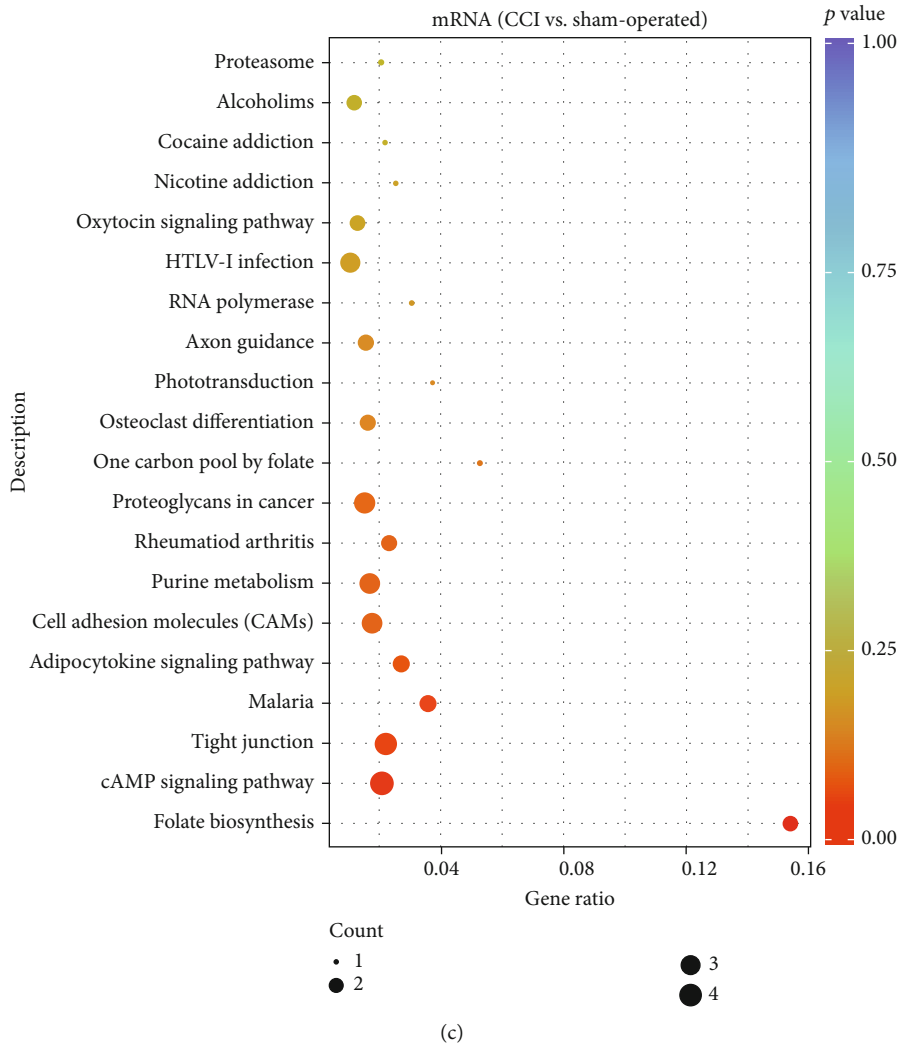

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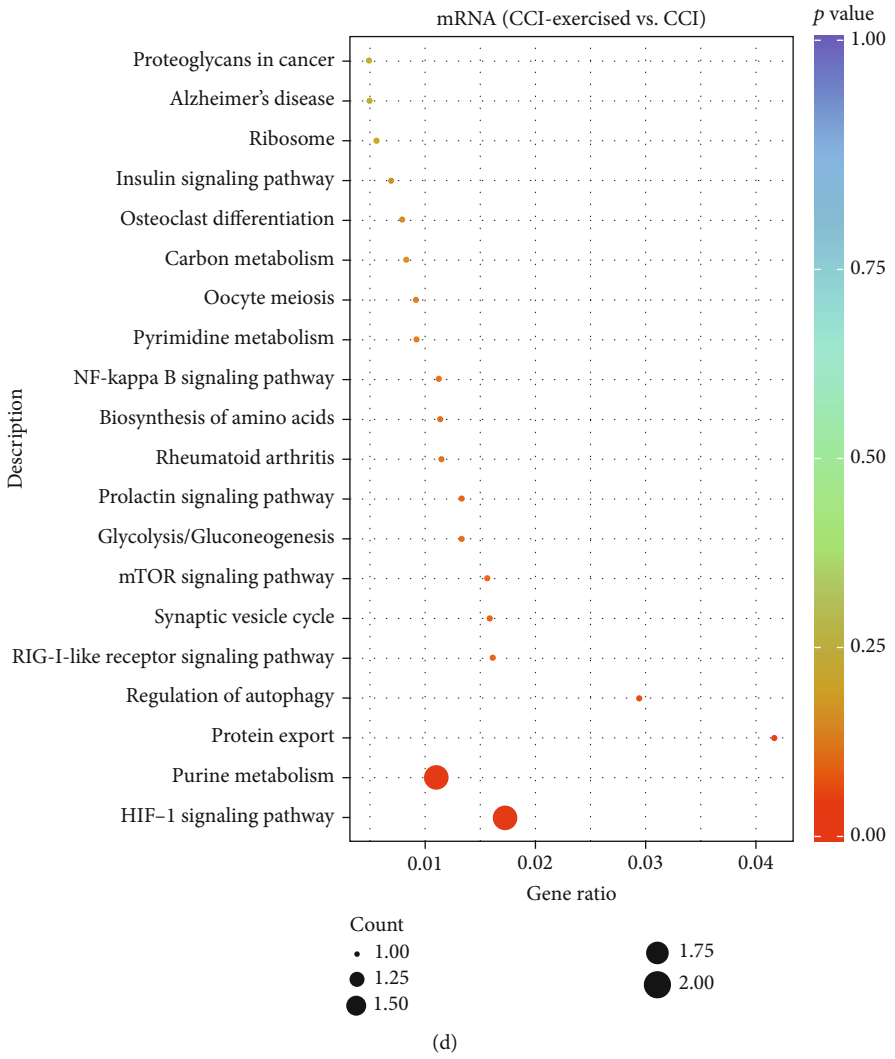
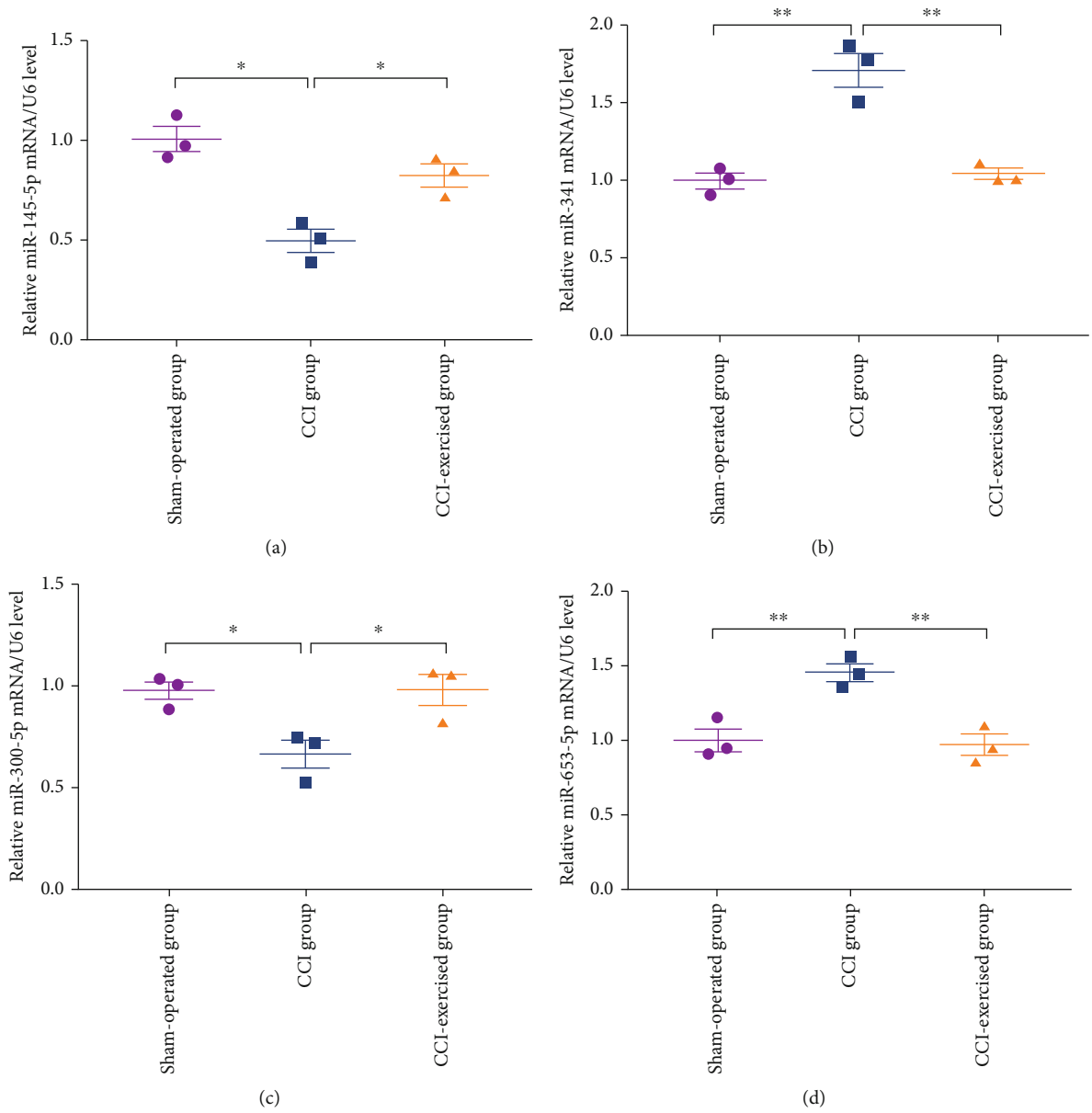


Figure 8: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway scatterplots of differentially expressed genes (DRGs). MicroRNA- (miRNA-) enriched KEGG pathway scatterplots showing statistics of pathway enrichment in comparisons of (a) the sham-operated group versus the chronic constrictive injury (CCI) group and (b) the CCI-exercised group versus the CCI group. mRNA-enriched KEGG pathway scatterplots showing statistics of pathway enrichment in comparisons of (c) the sham-operated group versus the CCI group and (d) the CCI-exercised group versus the CCI group. The size of the point represents the number of candidate target genes in the pathway, and the color of the point corresponds to different p value ranges ($n = 3$ animals per group).

activating GalR1 and GalR2 receptors. These studies suggest that Atf3, Ctss, and Cacna2d1 are pain inducers, and Gal was a pain protector. Previous studies and our transcriptome data confirmed that Atf3, Ctss, Cacna2d1, and Gal are significant upregulated after NP. Moreover, the expressions of Atf3, Ctss, Cacna2d1, and Gal were recovered after 4 weeks of exercise training, which may lead to improvements in mechanical allodynia and hyperalgesia in exercised CCI rats.

GO analysis revealed that signal transduction, defense response, and calcium channel activity are the main responses to peripheral nerve injury between the sham-operated and CCI groups. Additionally, between the CCI and CCI-exercised groups, defense response, voltage-gated calcium channel complex, kinase activator activity, postsynaptic endocytic zone, and methyltransferase activity were the main responses to exercise after CCI in DRG neurons. KEGG analysis revealed that the DEGs were significantly enriched in the classifications of Inflammatory mediator regulation of TRP channels, TNF signaling pathway, Rap1 signaling pathway, NF-kappa B signaling pathway, and MAPK signaling pathway in the comparison of the Sham-operated group versus the CCI group. After 4 weeks of exercise, the HIF-1 signaling pathway, Rap1 signaling pathway, T cell receptor signaling, B cell receptor signaling pathway, and neurotrophin signaling pathway were enriched. It is found that these pathways are closely related to the occurrence and development of NP through retrieving. For example, the TNF signaling pathway was significantly enriched in the sham-operated group compared with the CCI group. TNF superfamily cytokines represented a group of multifunctional proinflammatory cytokines that activated the signaling pathways of cell survival, apoptosis, inflammatory response, and



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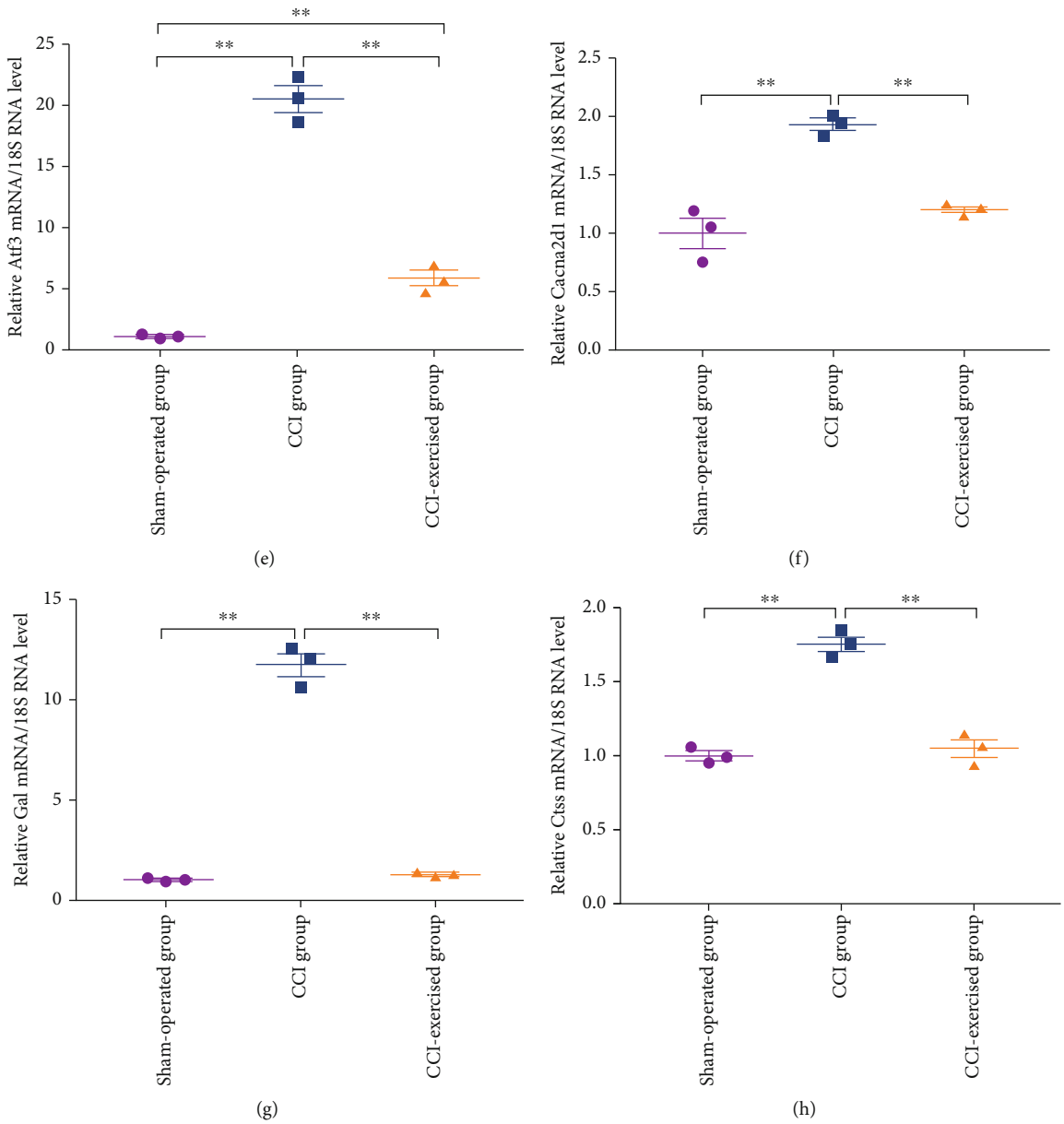

Figure 9: Validation of DEGs by quantitative real-time PCR (qRT-PCR). mRNA expression levels of (a) mir-145-5p, (b) mir-341, (c) mir-300-5p, (d) mir-653-5p, (e) Atf3, (f) Cacna2d1, (g) Gal, and (h) Ctss in the L4–L6 DRG at 28 days after surgery. Analyses of data were done by one-way ANOVA, followed by Tukey's multiple comparison test. Values indicate the mean ± standard error of mean; $n = 3$ animals/group. $*$ denotes $p < 0.05$; $**$ denotes $p < 0.01$.

cell differentiation. There is increasing evidence that TNF-α plays an important role in NP. Xu et al. showed that the injury of L5 nerve root transection can cause persistent mechanical allodynia and hyperalgesia in the hind paw of rats [49]. The immunoreactivity of TNF-α and its receptor 1 (TNFR1) in the damaged DRG increased significantly from 1 day after injury and lasted for 2 weeks. Immunofluorescence double staining showed the increase of TNF-α in satellite glia, microglia, and neurons, while TNFR1 only existed in DRG neurons. Preoperative intraperitoneal injection of TNF-α inhibitor can block mechanical allodynia and hyperalgesia. However, if it was used on the 7th day after the operation, the pain could not be reversed. These data suggest that the upregulation of TNF-α and TNFR1 in DRG and spinal dorsal horn is necessary for the initiation of NP caused by L5 nerve root transection, but they are not the key factors for subsequent pain maintenance [49]. Furthermore, the neurotrophin signaling pathway was enriched in the comparison of the CCI group versus the CCI-exercised group. The neurotrophins are composed of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), which mediated by the neurotrophin receptor p75NTR and the tropomyosin receptor kinases (Trks)

[50]. Atf3 has been found to be positive in 82% of L4 DRG neurons after sciatic nerve injury, but the positive rate decreased to 35% after the intrathecal delivery of NGF [51]. These results demonstrate that the expression of Atf3 may be induced by loss of NGF. Moreover, Geremia et al. [52] revealed that electrical stimulation increased BDNF expression in DRG neurons following peripheral nerve injury. The application of neurotrophins in NP shows that neurotrophins play an important role in peripheral nerve repair.

There are some limitations in this study. Firstly, the time point of 4 weeks after exercise was chosen on the basis of previous studies to observe expression changes of genes in response to exercise. However, in the process of behavioral analysis, it was found that mechanical hyperalgesia and thermal hyperalgesia were also significantly improved at 3 weeks. Therefore, the present study does not sufficiently reflect all important alterations in the DRG transcriptome after exercise. We recommend further research with more time points to address this limitation. Secondly, only male rats were used, and the potential sex dimorphic results are not reflected in our study. Estrogen can influence pain behaviors in rats [53]. Fan et al. [54] reported that the change of androgen level has no obvious effect on pain threshold, while ovarian hormone may inhibit the formation of mechanical hyperalgesia, but it had no obvious effect on the thermal pain threshold. We need to pay more attention to females in future research.

5. Conclusion

This study provides an understanding of the adaptive mechanisms after exercise of the DRG by transcriptional profiling. Multiple DEGs (such as miR-145-5p, miR-341, miR-300-5p, miR-653-5p, Atf3, Cacna2d1, Gal, and Ctss) were identified under pairwise comparisons of the sham-operated group versus the CCI group and the CCI group versus the CCI-exercised group. The differentially expressed miRNAs and mRNAs identified in this work may be new therapeutic targets for the treatment of NP induced by peripheral nerve injury. However, further miRNA interference experiments need to be carried out to confirm whether the target genes will change with the increase or decrease of expression of these differentially expressed miRNAs.

Data Availability

The datasets generated for this study have been deposited in the Sequence Read Archive database of NCBI, and the BioProject accession is PRJNA734377.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Supplementary Materials

Table S1: sequencing data quality summary of microRNAs (miRNAs). Table S2: sequencing data quality summary of mRNAs. *(Supplementary Materials)*

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Research Article

The Evaluation of Pain with Nociceptive and Neuropathic Characteristics from Three Different Perspectives in Amyotrophic Lateral Sclerosis Patients: A Case Controlled Observational Study in Southwestern China

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Background. Pain was considered a common and neglected symptom in amyotrophic lateral sclerosis (ALS) and had a substantial impact on the quality of life of ALS patients and their caregivers. However, pain in ALS was mainly evaluated from the perspective of nociceptive pain; only three studies referred to neuropathic pain in ALS, and there has been yet no study considering the neuropathic pain characteristics in ALS patients from China. Therefore, the purpose of our study was to determine characteristics of pain (nociceptive pain and neuropathic pain) by three different types of questionnaires. The correlation between pain and clinical parameters in ALS patients was also evaluated. *Methods*. Patients were eligible if they fulfilled the criteria of probable and definitive ALS according to the revised El Escorial criteria. Healthy normal controls, matched to ALS patients by age and gender, were recruited. Pain was evaluated by numerical pain rating scale (NRS), Brief Pain Inventory (BPI), and Douleur Neuropathique-4 (DN4) in ALS patients and controls. Physical status of ALS patients was evaluated with ALS Functional Rating Scale-revised (ALSFRS-R). *Results*. 65 patients with sporadic ALS and 100 healthy normal controls in Southwestern China were included. Pain in the preceding week was more frequently reported by patients with ALS (30, 46.2%) than controls (36, 36%) ($p = 0.193$). DN4 score $\geqslant 4$ was found in three ALS patients and one control ($p = 0.480$). Ten ALS patients (33.3%) and twenty-eight controls (77.8%) ($p < 0.001$) received therapy for pain. ALS patients with a DN4 score ≥ 4 had a longer disease duration and a higher PSI and PII score than ALS cases reporting nociceptive pain ($p = 0.041$, 0.048, and 0.027, respectively). Pain mainly interfered with ALS patients' mood, enjoyment of life, and the Pain Interference Index (PII) score. *Conclusions*. Our findings indicated that pain in our ALS cohorts was insufficiently treated and interfered with patients' mood and enjoyment of life. Most notably, we found that ALS patients with a DN4 score $\geqslant 4$ may have a longer disease duration and a higher PSI and PII score than ALS patients reporting nociceptive pain, which has never been reported, strongly deserving further validation.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of unknown etiology, characterized by the progressive loss of upper and lower motor neurons, causing weakness and atrophy of upper and lower limbs, dysphagia, and dysarthria, which eventually resulted in death due to respiratory failure typically within 2–4 years from onset [1]. Previously, pain has been considered relatively rare in ALS patients. Since an increasing number of studies about pain have been reported worldwide, pain was considered a common and neglected symptom in ALS, with the frequency of pain varying from less than 15% up to 85% [1–19]. Considering the substantial impact of pain on the quality of life of ALS

patients and their caregivers, the identification and evaluation of pain in ALS should not be disregarded. Guidelines for ALS treatment also reported that pain may be present in ALS patients and should be treated [20]. However, due to the scarcity of research data, pain in ALS was still frequently underestimated and insufficiently treated. In addition, to the best of our knowledge, pain in ALS was mainly evaluated from the perspective of nociceptive pain with various methods or questionnaires in different studies, including visual analog scale (VAS) [14], numerical pain rating scale (NRS) [5], Brief Pain Inventory (BPI) [2, 3, 7, 9, 10, 16, 19], Pain Detect Questionnaire [3, 7], Wong-Baker Faces Pain Rating Scale (WBS) [3, 7], and McGill Pain Questionnaire (MPQ) [3, 7]. Besides, there were only three studies about neuropathic pain in ALS from American, France, and Brazil populations by using Douleur Neuropathique-4 (DN4) or Neuropathic Pain Scale (NPQ) [10, 11, 15]. In China, pain characteristics in ALS patients ($n = 89$) was only assessed in a northern city of China, however, without evaluation of the characteristics of neuropathic pain. That is, up to now, there has been no study considering the neuropathic pain characteristics of ALS patients in China.

Therefore, the objective of our study was to determine prevalence, severity, site, type of pain (nociceptive pain and neuropathic pain), and its treatment, interference with activities by three different types of questionnaires (NRS, single-dimensional scale; BPI, multidimensional scale; DN4, neuropathic pain scale) in patients with sporadic ALS and healthy controls from Southwestern China. The correlation between pain and clinical parameters in ALS patients was also evaluated.

2. Participants and Methods

2.1. Participants. Patients were eligible if they fulfilled the criteria of probable and definitive ALS according to the revised El Escorial criteria [21]. Patients who had signs and symptoms of (frontotemporal) dementia were excluded. All consecutive ALS patients were seen and diagnosed by author Yanming Xu (an expert specialized in neurodegenerative diseases) from the Department of Neurology in our hospital.

Healthy normal controls, matched to ALS patients by age and gender and free of neurodegenerative diseases, were recruited.

2.2. Methods. First, pain was measured by a numeric rating scale (NRS), as single-dimensional scale, anchored as 0-no pain and 10-severe pain, to describe overall pain level. NRS score was treated as a continuous variable in the analysis.

Then, pain was evaluated using the Chinese version of the Brief Pain Inventory (BPI), a multidimensional scale [22]. BPI is a structured self-administered qualitative and quantitative questionnaire that provides basic information of pain in the last week, indicating the worst, least, and average perceived pain intensity as well as the pain perceived at the time of the interview (scale from 0, "no pain," to 10, "pain as bad that you can imagine"). BPI also gives information about the quality of pain, the type and site of pain, and the performed treatments. Patients are also asked to indicate the relief from pain during the last week because of pain treatment on a scale going from 0% (no relief) to 100% (complete relief). Lastly, BPI evaluates the interference of pain with seven daily functions (general activity, mood, walking ability, normal work, relation with other people, sleep, and enjoyment of life) (scale from 0, "does not interfere," to 10 "completely interferes"). However, because ALS causes the loss of walking ability and interferes with work, these two functions were not considered for the analyses. A Pain Severity Index (PSI) was derived by averaging the following pain severity items: worst and average pain and pain perceived at the time of the interview [3, 23]. Pain degree was defined as no pain ($PSI = 0$), mild pain ($1 \leq PSI \leq 3$), moderate pain ($4 \leq PSI \leq 6$), and severe pain ($7 \leq PSI \leq 10$). A Pain Interference Index (PII) was derived by averaging the interference of pain on daily functions [3, 23].

Lastly, pain was assessed by neuropathique–4 (DN4) questionnaire (Neuropathic Pain Diagnostic Questionnaire). It includes seven symptoms and three physical examination items. A score of 1 is given to each positive item and a score of 0 to each negative item. Respondents with a total score $\geqslant$ 4/10 are considered to have neuropathic pain [24].

Physical functional status of ALS patients was evaluated with ALS Functional Rating Scale-revised (ALSFRS-R), a 12-item scale assessing various physical functions potentially compromised in ALS. Each item is rated from 0 (worse) to 4 (best), corresponding to a total score ranging from 0 to 48, with higher scores indicating greater physical status and function [25]. Data including gender, age, age at onset, site of onset, and disease duration were collected from all ALS patients.

2.3. Statistical Analysis. Comparisons between normal distribution variables or nonnormal distribution variables were performed with Student's t-test or Mann–Whitney Test. Frequencies were compared with chi-square. Continuous data are presented as mean ± standard deviation or median/range, and categorical variables are presented as counts or percentages. Correlations were calculated using Pearson's or Spearman's coefficients. All tests were two-tailed. p value < 0.05 was considered significant. Analyses were performed with SPSS 25.0.

The study has been approved by the Ethical Committee of our institution, and written informed consent was obtained from each participant.

3. Results

3.1. Demographic Data. The ALS cohort included 65 patients, 39 males and 26 females, with a mean age of 52.6 years and a mean disease duration of 10 months at the time of the interview. The healthy control cohort included 100 subjects, 61 male and 39 females, with a mean age of 52.7 years. A comparison of demographic data between ALS patients and healthy controls is shown in Table 1.

3.2. Prevalence, Site, Description, Severity of Pain, and Treatment. Pain in the preceding week was more frequently reported by patients with ALS (30, 46.2%) than controls (36, 36%) ($p = 0.193$). Twenty-four (80%) of ALS patients with pain considered that the occurrence of pain was related

Table 1: Comparison between ALS patients and controls and clinical characteristics of patients with ALS.

	ALS patients ($n = 65$)	Controls ($n = 100$)	p value
Gender (female : male)	26 : 39	39 : 61	0.9
Age at interview, years (range)	52.6 (30-76)	52.7 (44-68)	0.5
Site of onset (spinal : bulbar)	52 : 13	—	—
Disease duration, months (range)	10 (2-72)	—	—
ALSFRS-R score (range)	40 (21-47)	—	—

ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale-revised.

to ALS. Both ALS patients and controls reported pain most frequently at the upper/lower limbs ($p = 0.015$), followed by the trunk. The most used adjectives to describe pain were "sore" (63.3%) and "distending" (63.3%) in ALS patients, while "stinging" (41.7%) and "sore" (36.1%) in controls, all with statistically significance between both groups ($p < 0.05$). In addition, seven ALS patients (23.3%) indicated "tearing" as descriptors, which was absent in controls.

Mean pain score of NRS was 3.1 (SD 1.9) in ALS patients and 4.6 (SD 2.2) in controls ($p = 0.005$). Mean PSI ratings were detected 3.0 (SD 1.7) in ALS patients and 2.9 (SD 1.3) in controls ($p = 0.688$). Seven patients (23.3%) and nine controls (25%) reported moderate pain ($4 \leq$ PSI ratings ≤ 6) ($p = 0.875$), while none subject was considered severe pain (PSI ≥ 7). Mean pain score of DN4 was 1.2 (SD 1.4) in ALS patients and 1.1 (SD 1.0) in controls ($p = 0.712$); three patients and one control were found to have a DN4 ≥ 4 score ($p = 0.480$). None of them had other pathological conditions (mostly diabetes mellitus, malignancy, paraproteinemia, or vasculitis) known as potential cause of neuropathic pain. They described neuropathic pain as spontaneous numbness, burning, painful cold, or pins-and-needle.

Ten ALS patients (33.3% of those with pain) and twenty-eight controls (77.8% of those with pain) ($p < 0.001$) received a therapy for pain. Five ALS patients (16.7% of those with pain) used pharmacological treatments to relieve pain; six patients (20%) adopted physical therapy for pain relief and only in one patient, with both therapies simultaneously. Nineteen controls (52.8%) used drug therapy or rehabilitation therapy for pain control, respectively; ten subjects adopted both treatment methods. Among ALS patients and controls, nonsteroidal anti-inflammatory drugs (NSAIDs) were the drugs more commonly used for treatment of pain. However, no ALS patients or controls were found to take nonopioid analgesics, opioids, or antiepileptic drugs. Physical therapy, acupuncture, and massage were the most common rehabilitation methods chosen by ALS patients and controls. Details about characteristics and therapy of pain in patients with ALS and controls are given in Table 2.

3.3. Clinical Characteristics of ALS Patients with Pain or without Pain. No differences were found in the genders, age at the time of the interview, and site of disease onset between ALS patients with nociceptive pain or without pain ($p = 0.455$, 0.539, and 0.534, respectively). Moreover, ALS patients with nociceptive pain or without pain had a similar disease duration and ALSFRS-R score at the time of the interview ($p = 0.663$ and 0.869, respectively).

Table 2: Characteristics and therapy of pain in patients with ALS and controls.

	ALS patients ($n = 65$)	Controls ($n = 100$)	p value
Reporting pain, n (%)	30 (46.2%)	36 (36%)	0.193
Localization of pain[a]			
Head/neck (%)	2 (6.7)	5 (13.9)	0.584
Trunk (%)	14 (46.7)	11 (30.6)	0.179
Upper/lower limbs (%)	28 (93.3)	25 (69.4)	0.015
Description of pain[a]		—	
Sore (%)	19 (63.3)	13 (36.1)	0.028
Distending (%)	19 (63.3)	9 (25)	0.002
Stinging (%)	3 (10)	15 (41.7)	0.004
Pain severity			
NRS (SD)	3.1 (1.9)	4.6 (2.2)	0.005
PSI (SD)	3 (1.7)	2.9 (1.3)	0.688
PSI ≥ 4, n (%)	7 (23.3)	9 (25)	0.875
DN4 (SD)	1.2 (1.4)	1.1 (1.0)	0.712
DN4 ≥ 4, n (%)	3 (5)	1 (1)	0.480
Treatment for pain			
Receiving treatment, n (%)	10 (33.3)	28 (77.8)	0.000
Drug therapy, n (%)	5 (16.7)	19 (52.8)	0.002
Rehabilitation therapy, n (%)	6 (20)	19 (52.8)	0.006

[a]Total is higher than 100% because more sites or description could be indicated. NRS: numerical pain rating scale; PSI: Pain Severity Index; DN4: Douleur Neuropathique-4; SD: standard deviation.

Age, ALSFRS-R score in ALS patients with DN4 score $\geqslant 4/10$ was not different from ALS cases only reporting nociceptive pain; however, ALS patients with a DN4 score $\geqslant 4$ seemed to have a longer disease duration and a higher PSI and PII score than ALS cases only reporting nociceptive pain ($p = 0.041$, 0.048, and 0.027, respectively) (data not shown).

3.4. Association of Pain with ALS Patients' Clinical Characteristics. There was a negative correlation between PSI score and ALSFRS-R score ($r = -0.398$, $p = 0.029$), while no significant correlation between the duration of the disease and PSI ratings was found ($r = 0.226$, $p = 0.23$) (data not shown).

Details about clinical characteristics of ALS patients with pain or without pain are shown in Table 3.

Table 3: Clinical characteristics of ALS patients with pain or without pain.

	ALS patients with pain ($n = 30$)	ALS patients without pain ($n = 35$)	p value
Gender (female : male)	13 : 17	12 : 23	0.455
Age at interview, year (SD)	51.7 (10.7)	53.1 (6.4)	0.539
Age at onset, year (SD)	50.9 (11.0)	52.5 (11.1)	0.568
Spinal onset, n (%)	25 (83)	27 (77)	0.534
Disease duration (range)	11 (2-26)	9 (2-72)	0.663
ALSFRS-R score (range)	41.5 (21-46)	38.97 (21-47)	0.869

ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale-revised; SD: standard deviation.

Table 4: Interference of pain on daily functions in all subjects with pain.

Pain interference item (range)	All subjects with pain		p value
	ALS patients ($n = 30$)	Controls ($n = 36$)	
General activity	0 (0-8)	0 (0-5)	0.171
Mood	1 (0-8)	0 (0-5)	0.002
Relation with other people	0 (0-8)	0 (0-5)	0.133
Sleep	0 (0-8)	0 (0-6)	0.071
Enjoyment of life	1.5 (0-8)	0 (0-7)	0.002
PII	1.6 (0-8)	0 (0-5)	0.013

PII: Pain Interference Index.

3.5. Pain Interference on Daily Functions. Pain mainly interfered with ALS patients' mood (median score of 1), enjoyment of life (median score of 1.5), and the summary score PII (median score of 1.6); other three domains of daily activities were relatively unaffected. The pain interference scores in those domains were significantly worse in ALS patients than in controls ($p = 0.002$, 0.002, and 0.013, respectively).

The areas of general activity, mood, and the summary score PII were correlated with PSI at $p < 0.05$ level in ALS patients cohort, especially in general activity ($p \leq 0.001$). Details about interference of pain on daily functions in all subjects with pain are shown in Table 4.

4. Discussion

Up to now, an increasing number of studies about pain in ALS patients from different countries worldwide have been conducted, with some conflicting results [2, 3, 5–17, 19].

In our case-control study, pain was slightly, but not significantly, more frequent in patients with ALS than in age- and gender-matched controls. The frequency of pain among ALS patients in previous studies varies greatly, from less than 15% up to 85% [1–11, 13–17, 19], which can be explained by the different study designs and settings, different populations and measure instruments or scales, and the number of ALS patients included (range from 7 to 424) [1]. Consistent with previous results, twenty-eight (93.3%) ALS patients in our cohort reported pain most frequently at the upper/lower limbs [3, 7, 15, 17], with a significant difference compared with controls. In a previous ALS-control study, only 10% of controls reported pain in the extremities; however, the number of controls involved in that study was small (46 controls) [7]. The most common types of pain were "sore" (63.3%) and "distending" (63.3%) in ALS patients, a little lower than that of previous results (85.7%, 40 ALS patients) [13]. However, seven patients (23%) described pain as "tearing," higher than that of previous results [13]. Both "sore" and "tearing" seemed to represent characteristics of secondary pain (mainly nociceptive), which developed in ALS patients as the disease progressed, whereby atrophy and weakness of muscles and prolonged immobility caused degenerative changes in connective tissue, bones, and joints leading to musculoskeletal pain [1].

Though mean pain score of NRS was a little higher in controls than in ALS patients, significant difference in PSI score between groups was not found, in consistent with the PSI score results in previous studies (patients 5.0 (SD 1.8) vs. controls 4.6 (SD 2.6); $p = 0.09$) (patients 3.0 (range 0.5–6.8) vs. controls 2.0 (range 0.5–5.3); $p = 0.08$) [3, 7]. Regarding NRS score was regarded as the pain perceived at the time of the interview, while PSI score was derived by averaging worst, average pain, and pain perceived at the time of the interview. So, the PSI score seemed to be more reasonable and representative for description of pain than NRS. Although severe pain (PSI ≥ 7) was absent in our ALS patient's cohort, seven ALS patients (23.3%) reported moderate pain ($4 \leq$ PSI ratings ≤ 6), which was in line with previous reports reporting moderate to severe pain in 14–36% of ALS patients [3, 13]. Lastly, only three ALS patients (5%) and one control (1%) presented with neuropathic pain (DN4 score $\geqslant 4/10$). Previously, only two studies have reported DN4 score $\geqslant 4/10$ in eight patients (8.6%) and one patient (1%) with ALS from France and Brazil, respectively [10, 11]. Our results further demonstrated ALS patients can have, though rarely, neuropathic pain characteristics.

In contrast to results of previous studies (47%, 70.3% ALS receiving treatment) [3, 7], our ALS patients with pain were undertreated, also less frequently treated than in controls. So, more attention should be paid to identify pain in patients with ALS and to treat it timely and appropriately. Only nonsteroidal anti-inflammatory drugs (NSAIDs), not nonopioid analgesics, opioids, or antiepileptic drugs, were used for the treatment of pain in ALS patients and controls, further providing evidence for the prevalence of nociceptive pain, not neuropathic pain [1]. Compared with previous reports (17%, 11.8%) about massage, acupuncture, and ultrasound for relieving pain [7, 19], physical therapy, acupuncture, and massage were more frequently used by our patients and controls (20% vs. 52.8%), suggesting those traditional rehabilitation therapies seemed more likely to be used by Chinese patients [1].

In line with previous reports, no differences were found in the genders and age at the time of the interview between ALS patients with or without pain [3, 9–11, 13, 14].

Although there was conflicting evidence whether the intensity of pain correlated with disease duration and functional impairment [3, 9–11, 13, 14], ALS patients with or without pain in our cohorts had a similar disease duration and ALSFRS-R score. A previous study found ALS patients with localized pain seemed to present with spinal symptoms at disease onset more frequently, with the bulbar area spared [17]. In our study, up to 83% of ALS patients with pain had a spinal onset, without statistical difference between ALS patients with or without pain, which had been reported in previous studies [11, 14]. Also, consistent with past reports [3, 7], PSI score was negatively correlated with ALSFRS-R score; however, no significant correlation between the duration of the disease and PSI ratings was found. Notably, in contrast with a previous study [11], ALS patients with a DN4 score $\geqslant 4$ had a longer disease duration and a higher PSI and PII score than ALS cases only reporting nociceptive pain, which have never been reported so far. Whether this reflected a special feature of pain in ALS patients during disease course deserved further study in larger populations and other different countries in the future.

Pain interfered especially with mood and enjoyment of life in ALS patients, and the general activity, mood, and the summary score PII were correlated with PSI in ALS patient's cohort, significantly in general activity, which have been reported before [3, 7, 19].

The first limitation of our study was the relatively small sample size of ALS patients involved ($n < 100$) and the cross-sectional design (instead of a long-term follow-up); therefore, we could not determine the course of pain over time as the disease progressed. Then, the assessment of pain in ALS was mainly limited to nociceptive and neuropathic pain; other causes of pain, including cramp, spasticity, and noninvasive ventilation, were not included in our study. Lastly, incidence of joint contracture, angle of motion at pain site, and neurophysiological examinations were not evaluated, which may be helpful for exploring the mechanism for pain in ALS patients.

5. Conclusions

In our study, we comprehensively evaluated pain characteristics and its treatment, interference with daily activities by NRS, BPI, and DN4 in sporadic ALS patients and healthy controls and the correlation with clinical parameters in ALS from Southwestern China, which was the first study about comprehensive evaluation of pain (especially including neuropathic pain assessment) from three different perspectives among ALS patients in China.

Our findings indicated that pain in our ALS cohorts was insufficiently treated and interfered with patients' mood and enjoyment of life. Every effort should be made to identify pain in patients with ALS and to treat it appropriately. Most notably, we found that ALS patients with a DN4 score $\geqslant 4$ may have a longer disease duration and a higher PSI and PII score than ALS patients reporting nociceptive pain, which has never been reported, strongly deserving further validation in larger samples of ALS patients and in other different countries.

Data Availability

The original data of the current study are available from the corresponding author on reasonable request.

Ethical Approval

The present study was approved by the Ethics Committee of West China Hospital of Sichuan University.

Consent

Written informed consents from the patients and controls have been obtained for participating in the study and publication of this paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ran An and Yan Li contributed equally to this work.

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Research Article

The Effect of Virtual Reality Training on Anticipatory Postural Adjustments in Patients with Chronic Nonspecific Low Back Pain: A Preliminary Study

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Objectives. This study is aimed at exploring the effects of virtual reality (VR) training on postural control, measured by anticipatory and compensatory postural adjustments (APAs and CPAs, respectively), in patients with chronic nonspecific low back pain (CNLBP) and the potential neuromuscular mechanism of VR training. *Methods*. Thirty-four patients were recruited and randomly assigned to the VR group ($n = 11$), the motor control exercise group (MCE, $n = 12$) and the control group (CG, $n = 11$). The VR group received VR training using Kinect Xbox 360 systems and magnetic therapy. Besides magnetic therapy, the participants in the MCE group performed real-time ultrasound-guided abdominal drawing-in maneuver (ADIM) and four-point kneeling exercise. The CG only received magnetic therapy. Surface muscle electromyography (sEMG) was used to record the muscle activities of transverse abdominis (TrA), multifidus (MF), lateral gastrocnemius (LG), and tibialis anterior (TA) during ball-hitting tasks. The muscle activation time and integrals of the electromyography activities (IEMGs) during the APA and CPA stages were calculated and used in the data analysis. The visual analogue scale (VAS) and Oswestry dysfunction index (ODI) scores were also recorded. *Results*. A significant interaction effect of time × group was observed on the activation time of TrA ($p = 0.018$) and MF ($p = 0.037$). The post-intervention activation time of the TrA was earlier in the VR group ($p = 0.029$). In contrast, the post-intervention activation time of the MF was significantly delayed in the VR group ($p = 0.001$). The IEMGs of TrA ($p = 0.002$) and TA ($p = 0.007$) during CPA1 significantly decreased only in the VR group after the intervention. The VAS scores of three group participants showed significant decreases after intervention ($p < 0.001$). *Conclusions*. Patients with CNLBP showed reciprocal muscle activation patterns of the TrA and MF muscles after VR training. VR training may be a potential intervention for enhancing the APAs of the patients with CNLBP.

1. Introduction

Chronic nonspecific low back pain (CNLBP) is one of the most common musculoskeletal disorders worldwide [1]. CNLBP may lead to a poor quality of life and increase the economic burden [2]. In recent years, altered postural control patterns have been reported as one of the most important factors that may contribute to CNLBP development [3, 4]. Postural control can be attributed to two different neuromuscular control mechanisms: anticipatory postural adjustments (APAs) and compensatory postural adjustments (CPAs). APAs are feed-forward adjustments that occur before perturbation to minimize the effects of predictable perturbations [5], whereas CPAs are reflexive adjustments made to maintain equilibrium after the onset of perturbations [6]. The central nervous system (CNS) employs APAs and CPAs to maintain stability. Previous studies have reported delayed or absent trunk muscle activation in rapid arm movement

or in response to sudden loading in patients with CNLBP [3, 7–9]. For instance, when a resistance force at the back was suddenly released, patients with chronic low back pain (CLBP) showed delayed activation time of abdominal muscles (transverse abdominis (TrA)) in the APA phase in comparison with healthy controls [9]. These findings were supported by the pain adaptation model, which postulates that long-term pain would decrease muscle activation when the muscle is activated as an agonist. Since upper limb loading produces a flexion moment, the TrA is the agonist during sudden upper limb loading and may show decreased electrical activity in the low back pain (LBP) group [9]. The impaired APAs in patients with CNLBP could activate trunk muscles in response to sudden perturbations over time and subsequently lead to the recurrence of LBP. Thus, a training program aimed at enhancing APAs is crucial for CNLBP patients.

Motor control exercise (MCE) is a common exercise rehabilitation program for patients with CNLBP [10]. However, the influence of MCE on APAs in patients with CNLBP is inconsistent. Several studies have reported that MCE could not enhance APA capacity [11–13], while Tsao and Hodges found that MCE can cause early muscle activation [14]. However, the muscle activation time after MCE was later than the time window between -100 and 50 ms, suggesting that MCE may not be a suitable rehabilitation training to enhance the APAs of CNLBP patients and that it may be more beneficial in enhancing postural stability by strengthening muscle power and endurance rather than increasing feed-forward control (e.g., APAs) by the CNS [11, 13].

Many studies have shown that virtual reality- (VR-) based training could improve participants' balance ability by providing multisensory feedback, for example, in chronic poststroke survivors [15, 16] and elderly people [17]. Two previous studies found that VR training could enhance APA capacity [18, 19]. Ida et al. found that when healthy participants lifted one foot to avoid a real or virtual obstacle, muscle activation of the supporting leg and trunk was observed during APAs in both real and VR environments, even though muscle activation in the virtual display setting was smaller than that in the real setting. In another study, PD (Parkinson's disease) participants showed shorter movement time and higher peak velocity of arm movements and longer APAs while catching a fast ball than they did while catching a slow ball in a VR environment. These results suggest that VR-based training can improve APAs. VR training has also been gradually applied to rehabilitation training programs in patients with CNLBP, and it has been shown to reduce pain and improve dysfunction [20]. However, the effect of VR training on APAs in patients with CNLBP is still unknown.

The present study is aimed at investigating the effect of VR-based training on APAs in patients with CNLBP by employing a ball-hitting test. We hypothesized that in comparison with MCE, VR-based training could enhance the APA capacity, including the muscle activation time and integrals of the EMG activities (IEMGs), in patients with CNLBP. The findings of the present study could help verify whether VR training is an effective treatment for enhancing postural control in patients with CNLBP.

2. Methods

2.1. Participants. Thirty-four right-handed participants with CNLBP were recruited for this study. The inclusion criteria for CNLBP participants were as follows: (1) age between 18 and 40 years, (2) persistent or periodic LBP for longer than 3 months, and (3) no referred symptoms of radiating pain below the knee or paresthesia during the straight-leg raise test. The exclusion criteria were as follows: (1) history of pelvic or spinal column surgery in the past two years; (2) diagnosis of any specific lumbar pathological condition (such as lumbar tumors, vertebral fractures, lumbar spinal stenosis, lumbar spondylolisthesis, rheumatoid arthritis, or ankylosis) and/or severe or progressive scoliosis; (3) body mass index (BMI) $\geq 30\,kg/m^2$; (4) history of a treatment program within the past three months; (5) pregnancy; (6) history of severe dysfunction of vital organs (heart, lungs, and kidneys) and/or cognitive deficits; and (7) history of visual or hearing problems. The participants could withdraw the experiment at any time if they (1) were unwilling to participate in this experiment, (2) felt any aggravation of pain during the treatment, (3) had any other disease due to the treatment, and (4) could not complete the proposed treatment plan. Ethical approval for this study (ethics: no. [2020]476) was obtained from the First Affiliated Hospital of Sun Yat-sen University. Written informed consent was obtained from all the participants prior to the experiment.

In our pilot study, the effect sizes (η^2_p) for the within factor (time) and within-between interaction (time × group) of muscle activation time were achieved in TrA muscle (0.113 and 0.244, respectively). G∗Power (v 3.1.9.7, Germany) was employed to calculate the sample size. 30 participants were required to achieve the statistical power in TrA.

2.2. Apparatus and Data Preprocessing

2.2.1. The Ball-Hitting Test. The participants stood at the center of a platform with their feet shoulder-width apart. They were asked to keep their elbows bent at 90° while holding a metal tray in their hands. A pressure sensor at the outer center of the base of the tray was used to determine the time point (T0) when the object landed on the tray. After a sound, a load weighing 1.5 kg was suddenly released by the experimenter from the participants' eye level above the tray (Figure 1). The participants were asked to try their best to maintain their body stability throughout the experiment. Each participant repeated five trials with a rest period of approximately 30 s between trials. Surface electromyography (sEMG) data were simultaneously recorded during the ball-hitting test. LabView 15.0.1 software (National Instruments, Austin, TX, USA) was used to simultaneously collect the data from the pressure sensor and the sEMG system at a frequency of 1000 Hz. Sufficient test-retest reliability for five trials in the ball-hitting test was observed in all the muscle activation time and IEMGs (intraclass correlation coefficient: 0.430~0.856).

2.2.2. Surface Electromyography (sEMG). An sEMG system (Myomonitor IV; Delsys, USA) with eight channels was used

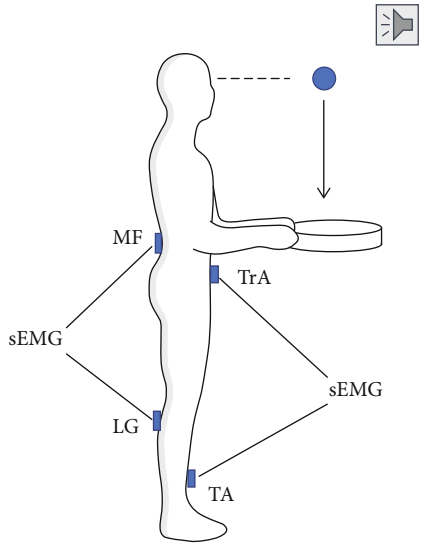

Figure 1: The setting of the ball-hitting test. sEMG: surface muscle electromyography; TrA: transverse abdominis; MF: multifidus; LG: lateral gastrocnemius; TA: tibialis anterior; VR: virtual reality training; MCE: motor control exercise.

to record muscle activity. The interelectrode distance of each channel was 10 mm. Before electrode attachment, the skin of the target areas was prepared by shaving, scrubbing with fine sandpaper, and rubbing with 75% alcohol to reduce impedance. Two electrodes were placed in a vertical arrangement along the muscle fibers of the transverse abdominis (TrA, 2 cm medial and inferior to the anterior superior iliac spine), multifidus (MF, at the level of the L5 spinous process on a line from the posterior superior iliac spine to the interspace between L1 and L2), lateral gastrocnemius (LG, at 1/3rd of the line from fibular head to the lateral side of the Achilles tendon insertion), and tibialis anterior (TA, at 1/3rd on the line between the tip of the fibula and the tip of the medial malleolus). The positions of the electrodes for these four muscles were based on previous studies [7, 8]. The reference electrode was placed on the patella on the dominant side. The sEMG data were sampled at a rate of 1000 Hz.

2.2.3. sEMG Data Preprocessing. The sEMG signals were processed using MATLAB software (The MathWorks Inc., Natick, MA, USA). The raw sEMG signals were rectified and bandpass-filtered (30–400 Hz). The first instance when the pressure signal acquired from the pressure sensor was equal to or greater than 5% of the peak magnitude for at least 20 ms continuously was considered as time zero (T0 = 0, the onset of a rise in the signal of the pressure sensor). The sEMG signals in the ball-hitting test were aligned using T0. The muscle activation time for each trial was detected in a time window from -300 ms to +200 ms in relation to T0. The previously described common methods for detecting muscle activation were not suitable in the present study because the low signal-to-noise ratio (SNR) of the sEMG signals increased the difficulty in detecting the muscle activation time. When the SNR of sEMG signals is very low, the onset time cannot be easily determined by visual inspection of the signal or by simply setting an amplitude threshold [21]. The TKE operation, which simultaneously considers the amplitude and instantaneous frequency of the surface EMG, has been previously used for detection of muscle activation time [21–23]. Thus, in the present study, the TKE operation was applied to determine the onset time of muscle activity. IEMGs were calculated in four epochs, each of 150 ms duration in relation to T0 [5]. The time windows for these four epochs were as follows: (1) from -250 ms to -100 ms (anticipatory reaction, APA1); (2) -100 ms to +50 ms (anticipatory reaction, APA2); (3) +50 ms to +200 ms (early compensatory adjustment, CPA1); and (4) +200 ms to +350 ms (late compensatory adjustment, CPA2). All IEMGs were corrected by the baseline IEMGs from -600 ms to -450 ms relative to T0. The mean muscle activation times and IEMGs were used in the following data analysis.

2.3. Interventions. All participants were randomly assigned to three groups: the VR, MCE, and control groups. Participants in the control group received conventional thermal magnetic therapy only, which was performed for 20 min with a medium heat level per day. In addition to thermal magnetic therapy, the participants in the VR group received VR training, whereas those in the MCE group performed MCE. All interventions were performed for two weeks, five days per week. More details regarding VR training and MCE are described below. Three physiotherapists responsible for intervention training were blinded to participants' outcome measures. Each of them took charge of the training program of one group. Two investigators were responsible for demographic information collection and outcome measures, respectively. These two investigators were blinded to the intervention allocation of each participant.

2.3.1. VR Training. The "Fruit Ninja" game displayed by the Kinect Xbox 360 system was employed for VR training. During the entire training session, the participant stood with feet shoulder-width apart at a distance of 1.5 m in front of the screen. The participants were asked to crush the fruit by waving their hands as much as possible, while simultaneously trying their best to avoid the "bombs" in the game (Figure 2). During training, the participants were asked to avoid trunk bending or turning. The participants needed to complete six sessions of VR training per day. Each session lasted three minutes, with a break of 2 min between sessions. It took approximately half an hour to complete the VR training per day.

2.3.2. Motor Control Exercise (MCE). The abdominal drawing-in maneuver (ADIM), which is a key technique in MCE training, was designed to enhance coactivation of the TrA and MF to stabilize the trunk before body movement. The ADIM in the present study was based on that used in a previous study [24]. Before the training, the participant learned how to specifically activate the TrA muscle under the guidance of real-time ultrasound without obvious contraction of the internal oblique and external oblique muscles simultaneously. When the participant could perform the

Figure 2: Example of VR training using Kinect Xbox 360 system.

ADIM appropriately, MCE training began. The first step of MCE involved three sets of ultrasound-guided ADIM training per day, with a short break of approximately 2 min between sets. Each set involved 10 repetitions of the ultrasound-guided ADIM, each of which lasted for 10 s. In the second step, the participant completed a four-point kneeling exercise. In the second step, the participant completed a four-point kneeling exercise. In the first stage, the participant was instructed to lift one arm with the elbow and wrist extended for 5 s and maintain the TrA contraction at the same time. Each side of the upper limb was repeated thrice with a break of 15 s. In the second stage, the participant lifted one leg with the hip and knee extended for 5 s in a four-point kneeling position. Each side of the leg was repeated thrice with a break of 15 s. In the last stage, the participants raised one arm and the contralateral leg to a horizontal position (bird dog) and held it for 5 s in a four-point kneeling position. Each movement was repeated three times with a break of 15 s. In the last stage, the participants raised one arm and the contralateral leg to a horizontal position (bird dog) and held it for 5 s in a four-point kneeling position. Each movement was repeated three times with a break of 15 s. Each participant was instructed by an experienced physiotherapist during the MCE training. The MCE training required approximately 30 min per day after conventional thermal magnetic therapy.

2.4. Procedure. Before the training, the participants completed a demographic information questionnaire assessing gender, height, weight, age, and medical history. A visual analogue scale [25] (VAS) (anchored with "painless" at 0 and "intolerable pain" at 10) was used to measure pain intensity, and the Oswestry disability index (ODI) [26] was used to assess function disability pre- and posttraining. The ball-hitting test with sEMG recording was also conducted pre- and postintervention. VR-based training and MCE training were performed after conventional thermal magnetic therapy. The experiment flow is shown in Figure 3.

2.5. Statistical Analysis. An independent t-test was used to compare between-group differences in all demographic variables except sex (Table 1). The chi-squared test was used to compare between-group differences in sex. The muscle activation times and IEMGs of the four target muscles as well as data from the clinical assessments (including VAS and ODI scores) were analyzed using the two-way mixed-design repeated-measure analysis of variance (ANOVA) with a between-subject factor of group (CG, MCE, and VR groups) and a within-subject factor of time (pre- and posttraining). Post hoc pairwise comparisons were applied when a significant effect was observed. The significance level was set at $p < 0.05$. SPSS software (version 23.0; IBM, Armonk, NY, USA) was used for data analysis.

3. Results

3.1. Demographics and Clinical Assessments. The demographic information of all participants is shown in Table 1. No between-group differences were found in sex, age, weight, height, BMI, or pain duration ($p > 0.050$) (Table 1).

3.2. Muscle Activation Time. The activation times of TrA, MF, LG, and TA are presented in Figure 4. A significant main effect of time was observed on the MF activation time ($F(1, 31) = 9.438$, $p = 0.004$, $\eta^2_p = 0.233$). A significant interaction effect of time × group was observed on the activation time of TrA ($F(2, 31) = 4.606$, $p = 0.018$, $\eta^2_p = 0.029$) and MF ($F(2, 31) = 3.662$, $p = 0.037$, $\eta^2_p = 0.191$). Post hoc analysis for the significant interaction effect revealed that the activation time of TrA after VR training was significantly earlier than that before training ($p = 0.029$). However, the activation time of the MF muscle in the VR group after training was significantly delayed ($p = 0.001$) in comparison with that before training. No changes in the activation times of TrA and MF were observed in the MCE (TrA: $p = 0.878$; MF: $p = 0.832$) and control groups (TrA: $p = 0.055$; MF: $p = 0.243$). Other main effects of time and group and the interaction effect of time × group were not significant ($p > 0.050$).

3.3. IEMGs of the Four Muscles. The IEMGs in the APA1 and APA2 phases are presented in Figures 5 and 6, respectively. The F ratios and p values for the mixed model of the four muscles during APA1 and APA2 are shown in Table 2. A significant effect of time was observed on the IEMGs of MF ($F(1, 31) = 5.226$, $p = 0.029$, $\eta^2_p = 0.144$) and TA ($F(1, 31) = 6.404$, $p = 0.017$, $\eta^2_p = 0.171$) during APA1

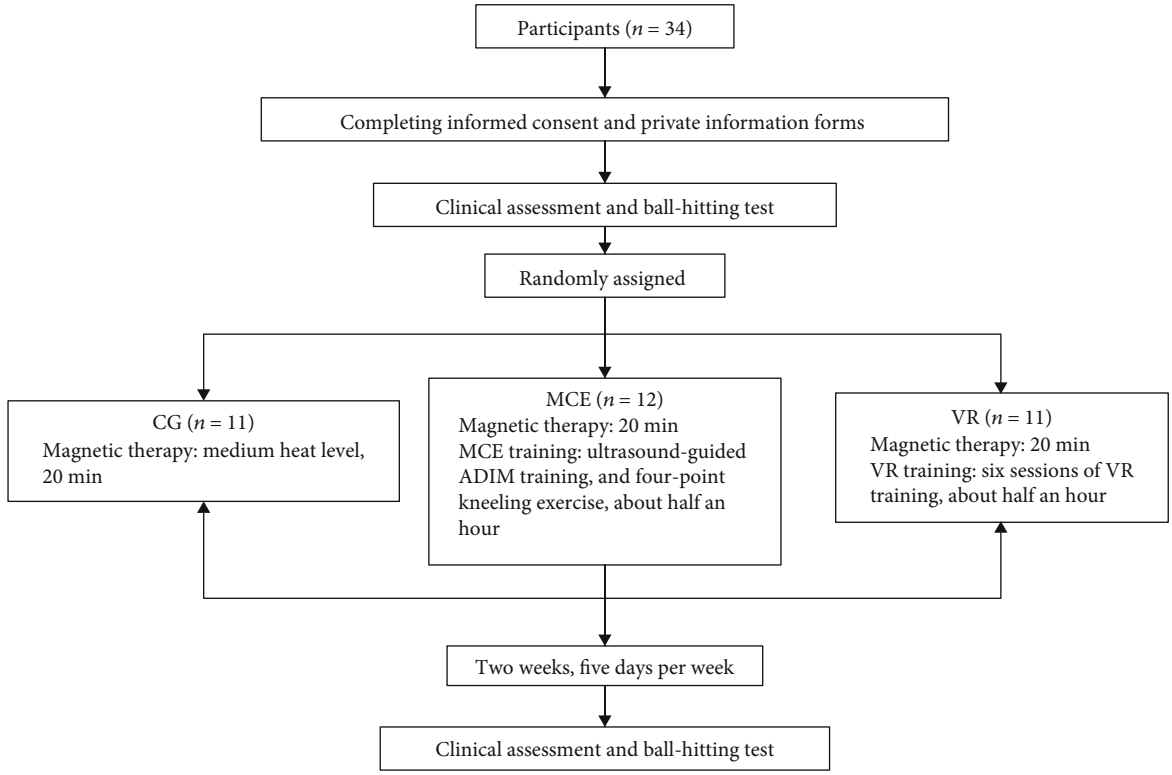

Figure 3: The experiment flowchart. VR: virtual reality training; MCE: motor control exercise; CG: control group; ADIM: abdominal drawing-in maneuver.

Table 1: Demographic information of the three groups of participants.

	CG ($n = 11$)	MCE ($n = 12$)	VR ($n = 11$)	p
Sex (male/female)	4/7	2/10	3/8	0.563
Age (years)	25.36 ± 3.72	23.75 ± 4.09	21.91 ± 2.43	0.085
Weight (kg)	61.82 ± 9.21	58.58 ± 12.29	58.01 ± 13.29	0.715
Height (m)	1.66 ± 0.07	1.67 ± 0.09	1.67 ± 0.07	0.850
BMI (kg/m^2)	22.33 ± 2.41	20.70 ± 3.034	20.44 ± 3.54	0.295
Pain duration (months)	49.82 ± 83.49	38.83 ± 37.20	30.18 ± 19.85	0.693

Notes: (1) values are mean ± SD; n represents sample size; (2) VR: virtual reality training; MCE: motor control exercise; CG: control group; BMI: body mass index.

and on the IEMGs of MF ($F(1, 31) = 8.344$, $p = 0.007$, $\eta^2_p = 0.212$) and TA ($F(1, 31) = 0.372$, $p = 0.027$, $\eta^2_p = 0.148$) during APA2. We also observed a significant effect of group on the IEMGs of LG ($F(1, 31) = 4.243$, $p = 0.024$, $\eta^2_p = 0.215$) during APA1. A significant time × group interaction effect was only observed on the TA during APA2 ($F(2, 31) = 11.514$, $p < 0.001$, $\eta^2_p = 0.426$). Other main effects of time and group or the interaction effect of time × group were not significant ($p > 0.050$). Post hoc analysis for the significant interaction effect showed that the IEMGs of TA during APA2 were significantly decreased after the intervention only in the VR group ($p < 0.001$), which could not be observed in the other two groups.

The IEMGs in the CPA1 and CPA2 phases are presented in Figures 7 and 8, respectively. The F ratios and p values for the mixed model of the four muscles during CPA1 and CPA2 are shown in Table 3. A significant effect of time was observed on the IEMGs of MF ($F(1, 31) = 4.256$, $p = 0.048$, $\eta^2_p = 0.121$), LG ($F(1, 31) = 6.907$, $p = 0.013$, $\eta^2_p = 0.182$), and TA ($F(1, 31) = 4.589$, $p = 0.040$, $\eta^2_p = 0.129$) during CPA1. A time × group interaction effect was observed on the IEMGs of TrA ($F(2, 31) = 6.409$, $p = 0.005$, $\eta^2_p = 0.293$) and TA ($F(2, 31) = 4.103$, $p = 0.026$, $\eta^2_p = 0.209$) during CPA1. Other main effects of time and group and the interaction effect of time × group were not significant ($p > 0.050$). Post hoc analysis for the significant interaction effect showed that the IEMGs of TrA ($p = 0.002$) and TA ($p = 0.007$) during

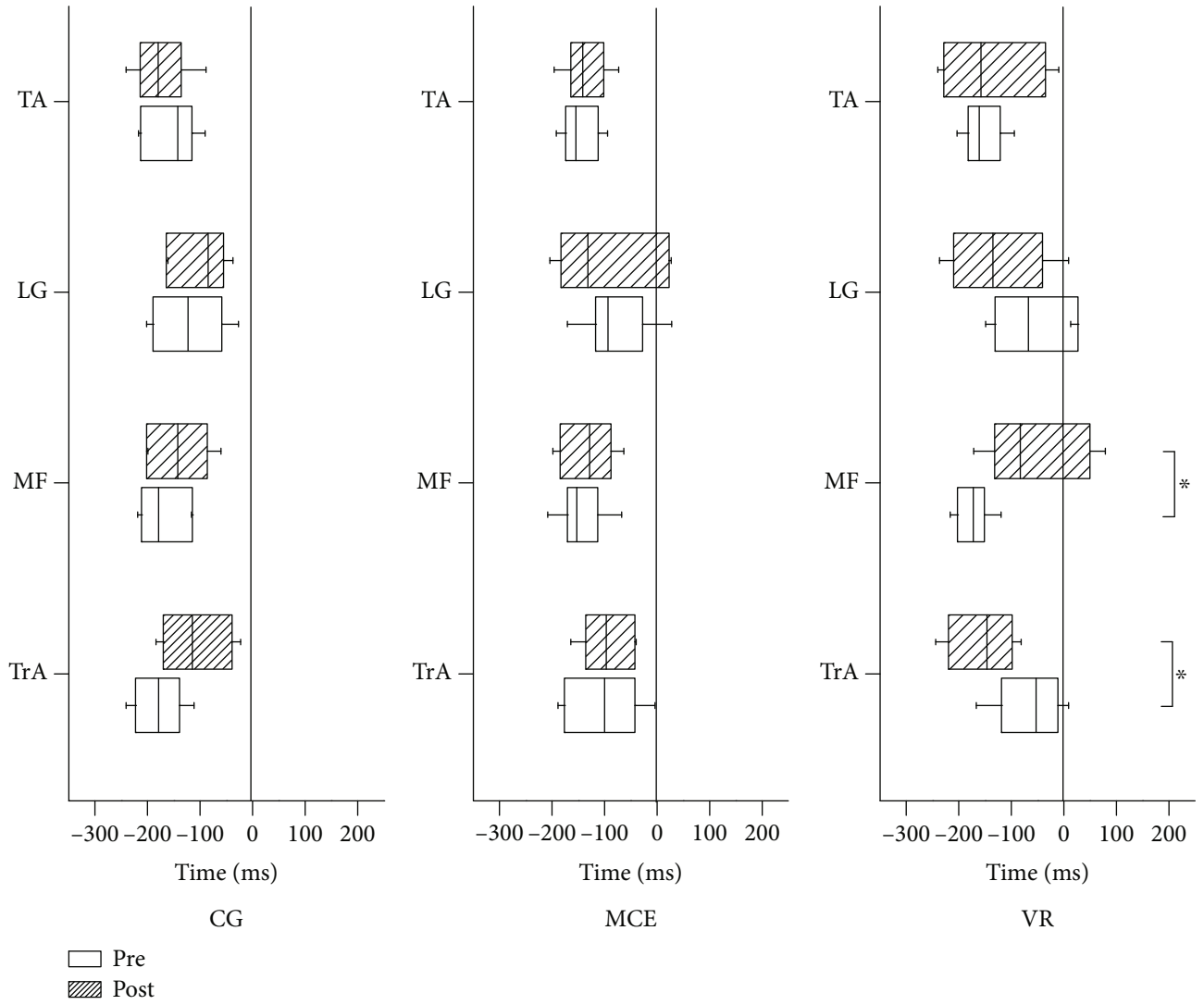

Figure 4: The mean muscle activation times of the four muscles pre- and posttraining for the three groups of participants. TrA: transverse abdominis; MF: multifidus; LG: lateral gastrocnemius; TA: tibialis anterior; VR: virtual reality training; MCE: motor control exercise; CG: control group. $^{*}p < 0.050$. Standard deviation bars are shown.

CPA1 significantly decreased after the intervention only in the VR group, which could not be observed in the other two groups.

3.4. Pain-Related Clinical Outcomes. Pain-related clinical outcomes, including VAS and ODI scores, are shown in Table 4. A significant main effect of time was observed on the VAS score ($F(1, 31) = 39.65$, $p < 0.001$, $\eta^2_p = 0.561$). The main effect of group ($F(2, 31) = 1.26$, $p = 0.298$, $\eta^2_p = 0.075$) and the time × group interaction effect ($F(1, 31) = 2.013$, $p = 0.151$, $\eta^2_p = 0.115$) were not significant for the VAS score. Time ($F(1, 31) = 1.70$, $p = 0.203$, $\eta^2_p = 0.052$), group ($F(2, 31) = 1.19$, $p = 0.317$, $\eta^2_p = 0.071$), and time × group ($F(1, 31) = 0.023$, $p = 0.978$, $\eta^2_p = 0.001$) did not show significant effects on ODI scores.

4. Discussion

The present study investigated the effect of VR training on postural control in patients with CNLBP through measurement of APAs and CPAs in an external postural perturbation task. A novel finding was that the TrA muscle showed earlier muscle activation in APAs after VR training, whereas the MF muscle showed delayed activation after VR training. These findings were not observed in the other two groups. These findings suggest that VR-based training is likely to improve the APAs of CNLBP patients.

Postural control requires the central nervous system (CNS) to receive and integrate multisensory inputs (including vision, vestibular sense, and proprioceptive and tactile information) and thereby coordinate and control the postural muscles to maintain balance and stability of the body [27]. Impaired postural control has been reported to involve

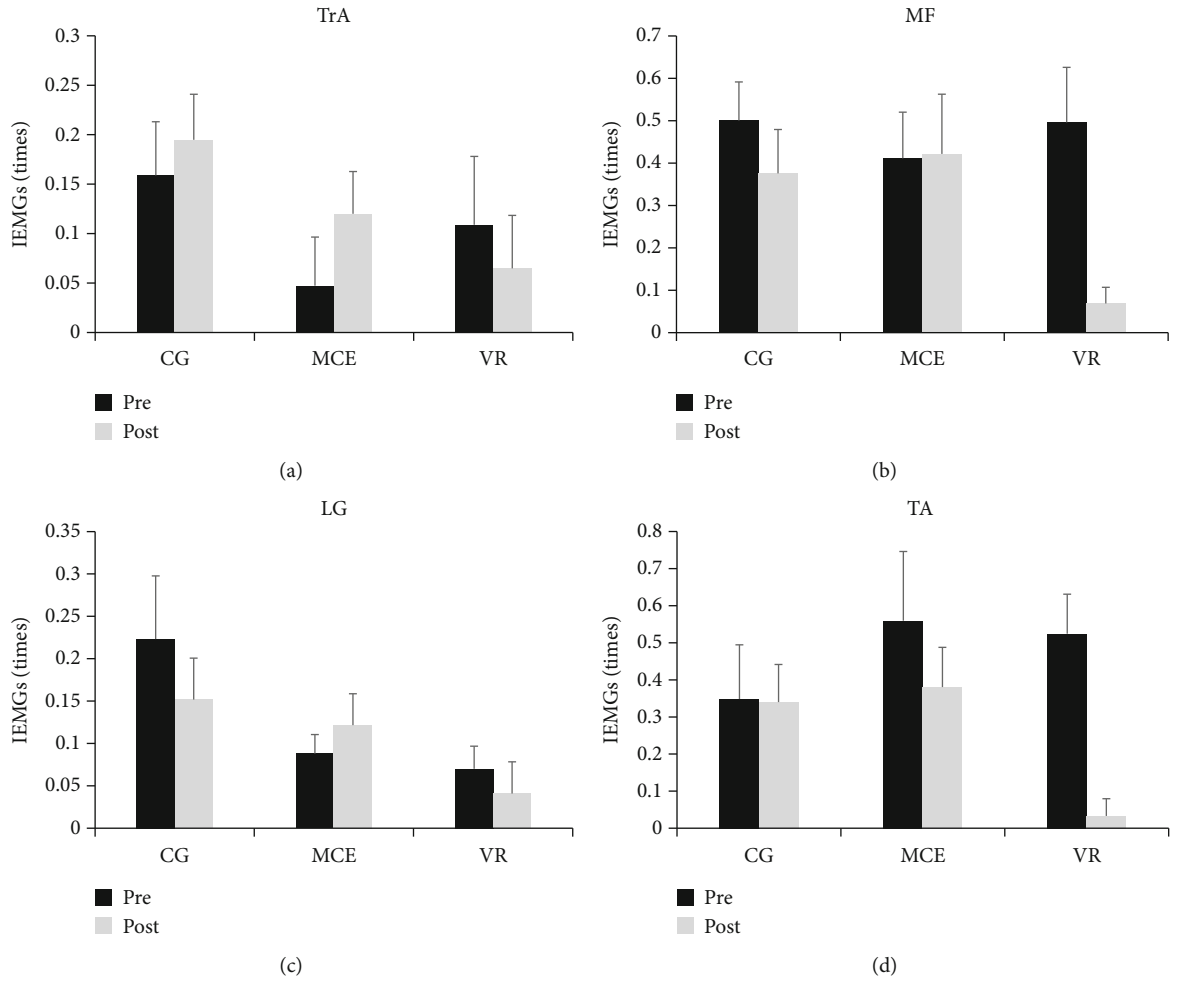

Figure 5: The pre- and posttraining IEMGs of the four muscles during APA1 in the three groups of participants. TrA: transverse abdominis; MF: multifidus; LG: lateral gastrocnemius; TA: tibialis anterior; VR: virtual reality training; MCE: motor control exercise; CG: control group. $^{*}p < 0.050$. Standard error of the mean bars are shown.

APAs and CPAs of trunk muscles [28, 29]. Most previous studies reported that delayed activation of abdominal muscles, especially TrA, in patients with CNLBP was commonly observed in patients with LBP in the postural control assessment, which may increase the recurrence of LBP in patients with CNLBP [4, 30, 31]. The potential reason was that the delayed muscle activation time of TrA in the APAs of patients with CNLBP was associated with remodeling of the motor cortex [32]. Sadeghi et al. found that the prefrontal cortex is activated in patients with LBP during postural interference [33], suggesting that the prefrontal and motor cortices are involved in anticipatory processing.

In this study, the muscle onset time of TrA in CNLBP patients through two weeks of VR exercise training was significantly earlier than that before training, suggesting that VR training could improve APAs. These findings are supported by the results of previous studies [18, 19]. For example, Su et al. showed that training for a ball-catching task in a VR environment could improve PD patients' ability to perform APAs. The possible mechanism is likely that VR training enhanced the activation of the frontoparietal and sensorimotor networks by providing visual cues and visual feedback to patients [34, 35]. The information provided by the virtual environment can increase the activation of the frontal lobe, which participates in the anticipatory process [34]. The anticipation process is a top-down cognitive process [33, 34], the preparatory state of which could be comparable to that during APAs [35]. Thus, in the present study, VR training could improve the APA capacity of CNLBP patients because the visual cues in the dynamic environment of "Fruit Ninja" elicited the participants' anticipation of the movement in response to the objects in the virtual environment. In addition, the "Fruit Ninja" game required the participant to move the arm rapidly in different directions to cut the fruits. Hodges reported that healthy participants would activate TrA muscle earlier than other muscles to maintain postural stability, when performing the rapid shoulder flexion, abduction, extension [36]. The role of TrA muscle in the arm movement in these studies was like to increase the stiffness of the lumbar spine through raising the intra-abdominal pressure and increase the tension of thoracolumbar fascia. Thus, the participants were required to activate TrA earlier to maintain postural stability during the arm movement of the "Fruit Ninja" game.

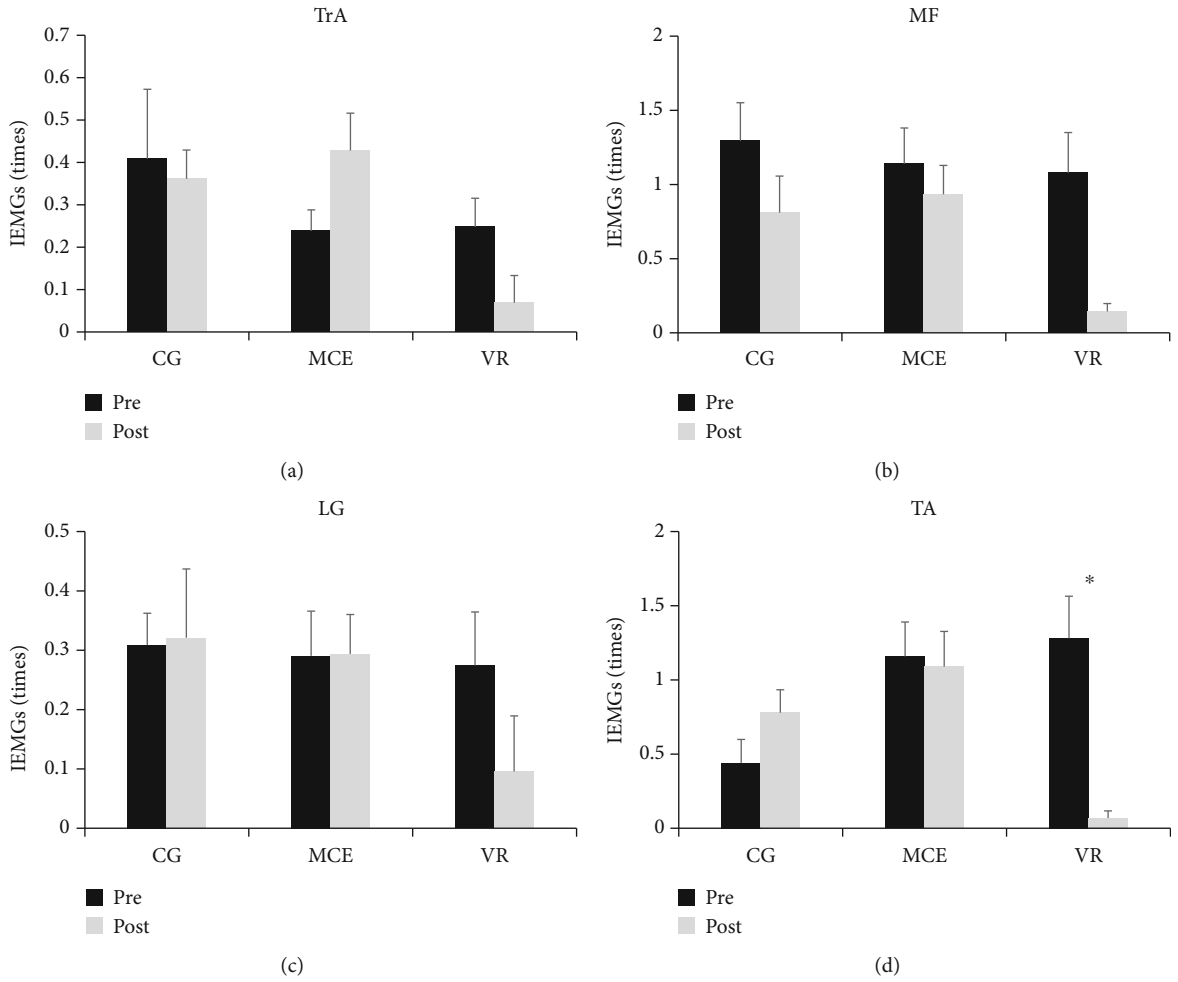

Figure 6: The pre- and posttraining IEMGs of the four muscles during APA2 in the three groups of participants. TrA: transverse abdominis; MF: multifidus; LG: lateral gastrocnemius; TA: tibialis anterior; VR: virtual reality training; MCE: motor control exercise; CG: control group. $^{*}p < 0.050$. Standard error of the mean bars are shown.

Table 2: Results of two-way mixed-design ANOVA for IEMGs of the four muscles in APA1 and APA2.

Muscle	APA1						APA2					
	Time		Group		Time × group		Time		Group		Time × group	
	F value	*p* value	*F* ratio	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value
TrA	0.382	0.541	1.251	0.300	0.851	0.437	0.021	0.886	2.406	0.107	2.713	0.082
MF	5.226	0.029	0.805	0.456	2.739	0.080	8.344	0.007	2.128	0.136	1.387	0.265
LG	0.259	0.614	4.243	0.024	0.659	0.524	0.338	0.565	1.106	0.343	0.471	0.629
TA	6.404	0.017	0.918	0.410	2.236	0.124	5.372	0.027	2.585	0.092	11.514	<0.001

Note: TrA: transverse abdominis; MF: multifidus; LG: lateral gastrocnemius; TA: tibialis anterior.

A previous study reported opposite muscle activation patterns of TrA (delayed activation) and MF (earlier activation) in patients with CNLBP [36]. This reciprocal activation pattern in the TrA-MF pair may be related to an efficient strategy for postural control by the CNS when the perturbation is predictable [37]. The present study showed that the activation of MF was delayed after two weeks of VR training. These results suggest that after VR training, the activation pattern of the MF muscle appears to be similar to that of the control participants.

This study also found that muscle activity of the TA during APA2 was weakened after VR training. A previous study reported that patients with CLBP adopted a body-and-trunk–stiffening strategy and relied more on ankle proprioception to control their posture while standing due to the weakness of their trunk muscles [38]. In this study, the IEMGs of the TA during APA2 decreased after VR training, possibly due to the improvement in TrA muscle activation after VR training. Thus, the improvement in the TrA muscle may enable CLBP patients to achieve better coordination of

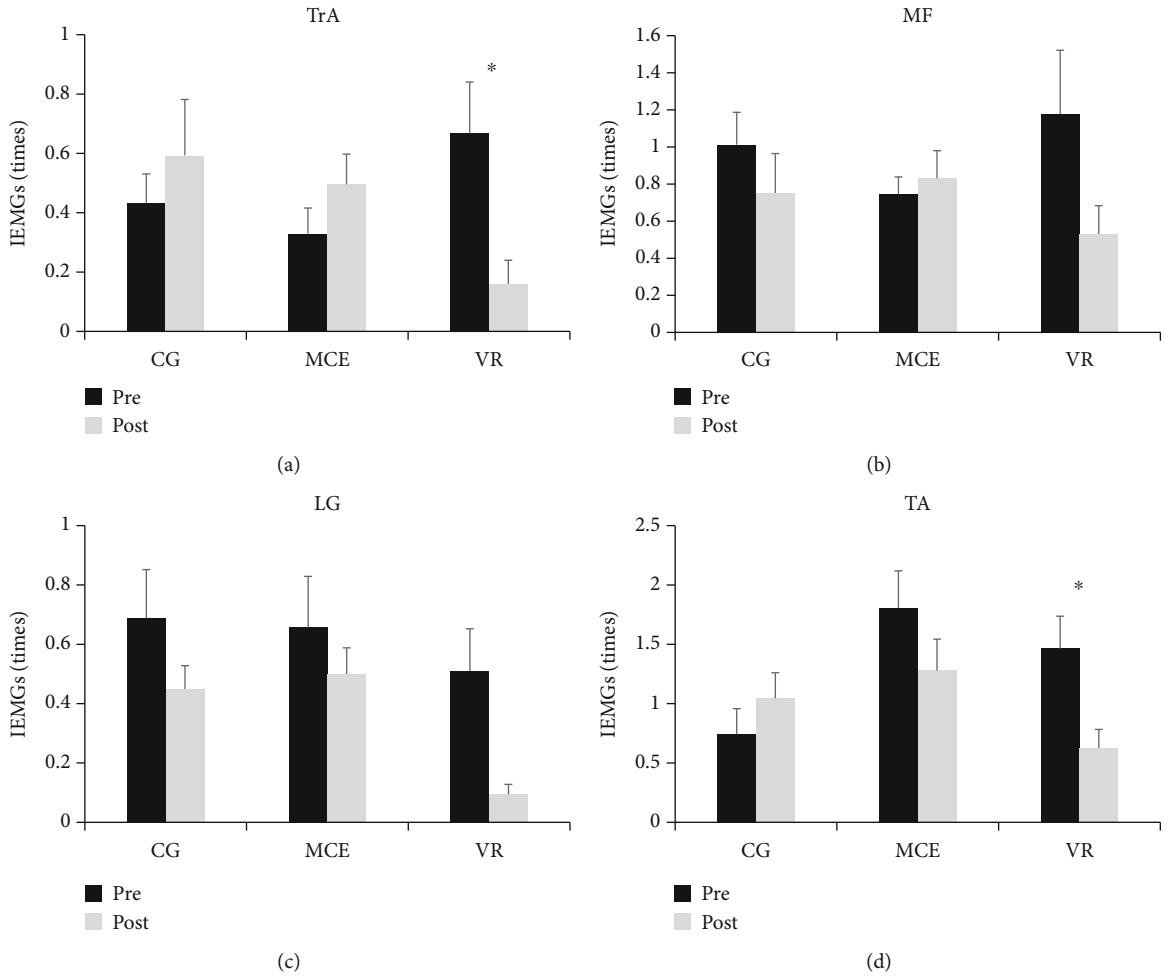

Figure 7: The pre- and posttraining IEMGs of the four muscles during CPA1 in the three groups of participants. TrA: transverse abdominis; MF: multifidus; LG: lateral gastrocnemius; TA: tibialis anterior; VR: virtual reality training; MCE: motor control exercise; CG: control group. $^{*}p < 0.050$. Standard error of the mean bars are shown.

the deep trunk muscle to maintain stability and rely less on the ankle strategy.

The present study also found that the muscle activities of the TrA and TA in the CPA1 stage were weakened after VR training. These results suggest that patients with CLBP tend to use the APA strategy to reduce the compensatory response of the muscle to external interference after VR training. These findings are consistent with those reported by Santos et al. [5] and Liang et al. [37]. Santos et al. found that when perturbations were predictable, stronger APAs were significantly related to smaller compensatory activities of muscles and COP displacements in response to external perturbation. The findings in Liang et al.'s study showed that after auditory training, the participants demonstrated stronger APAs and less demands on CPAs. In the present study, the IEMGs of TrA and TA of CNLBP participants decreased in CPA1 after VR training, which may result from the APA improvement due to the prediction elicited by the visual information in the virtual environment.

The muscle activation times and IEMGs showed no changes pre- and posttraining in the MCE and control groups. These results were supported by the findings reported by Vasseljen et al. [11] and Lomond et al. [12]. Vasseljen et al. found no significant difference in the activation time of abdominal muscles in patients with CLBP between pretraining and after 8 weeks of MCE training. A planned secondary analysis conducted by Lomond et al. revealed that low back stabilization or movement system impairment treatments did not ameliorate the CLBP participants' APAs impairment, as reflected by the lack of significant effects of treatment on IEMGs. This may be because MCE can enhance postural stability mainly by strengthening muscle power and endurance [12, 13]. However, APAs rely more on brain activities, including motor planning and the excitability of the motor cortex, to predict external perturbations [35, 39]. Thus, in the present study, MCE and conventional physiotherapy did not improve muscle activation time in patients with CNLBP, because the activities in these two groups did not require the participants to perform accurate predictions during the intervention.

As for the clinical outcomes, the VAS findings suggested that LBP decreased after training in the three groups of

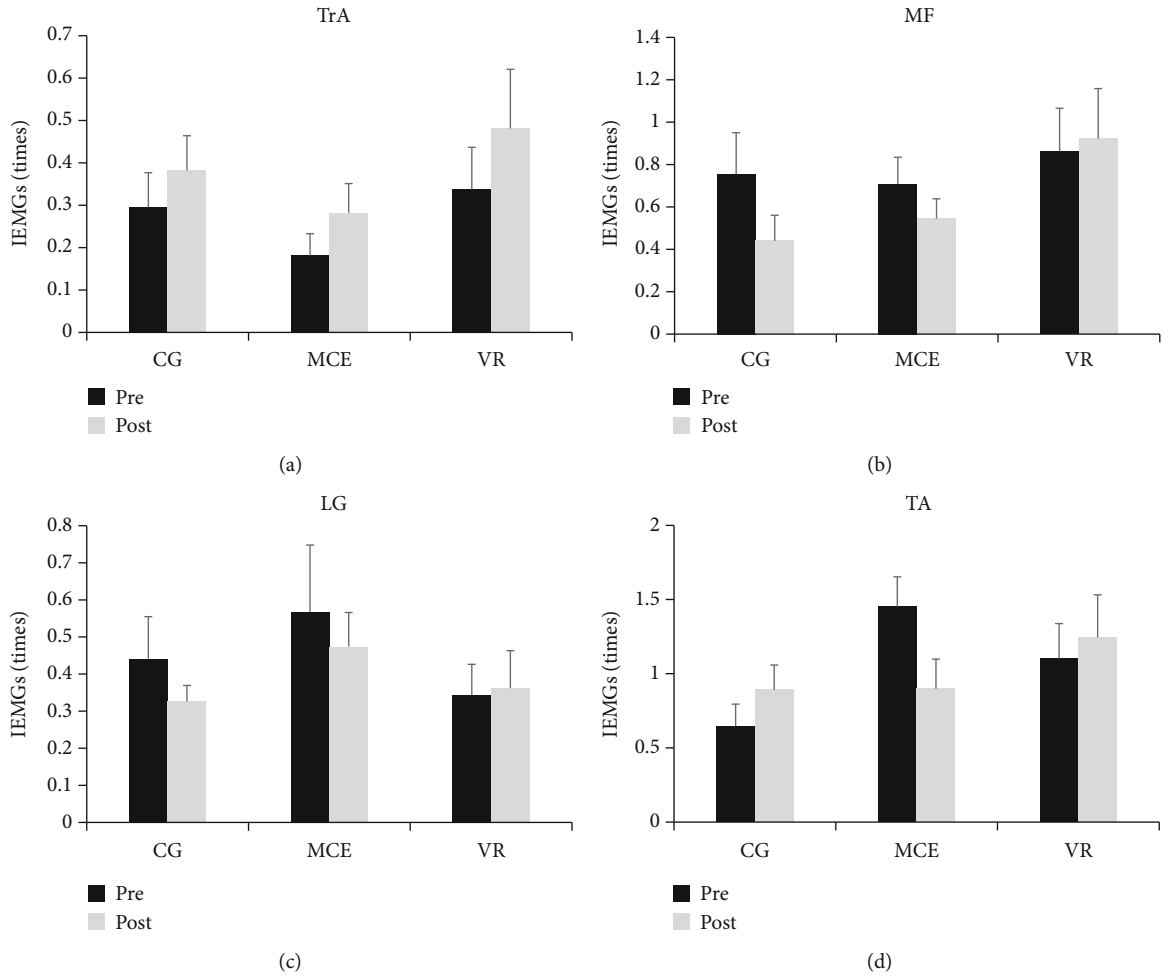

Figure 8: The pre- and posttraining IEMGs of the four muscles during CPA2 in the three groups of participants. TrA: transverse abdominis; MF: multifidus; LG: lateral gastrocnemius; TA: tibialis anterior; VR: virtual reality training; MCE: motor control exercise; CG: control group. $^{*}p < 0.050$. Standard error of the mean bars are shown.

Table 3: Results of two-way mixed-design ANOVA for IEMGs of four muscles in CPA1 and CPA2.

Muscle	CPA1						CPA2					
	Time		Group		Time × group		Time		Group		Time × group	
	F value	*p* value	*F* ratio	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value
TrA	0.599	0.445	0.251	0.780	6.409	0.005	3.474	0.072	1.959	0.158	0.078	0.925
MF	4.256	0.048	0.149	0.862	2.586	0.091	1.113	0.300	2.260	0.121	0.619	0.545
LG	6.907	0.013	2.575	0.092	0.583	0.564	0.601	0.444	1.003	0.379	0.151	0.860
TA	4.589	0.040	2.642	0.087	4.103	0.026	0.130	0.721	1.934	0.162	2.727	0.081

Note: TrA: transverse abdominis; MF: multifidus; LG: lateral gastrocnemius; TA: tibialis anterior.

Table 4: The pain-related clinical outcomes in the three groups of participants.

Test	VAS (mean ± SD)			ODI (mean ± SD)		
	CG	MCE	VR	CG	MCE	VR
Pre	3.64 ± 1.36	4.58 ± 1.83	4.36 ± 1.36	12.72 ± 4.84	18.42 ± 9.36	15.65 ± 6.39
Post	2.18 ± 1.17	2.17 ± 1.90	3.18 ± 1.08	9.63 ± 7.20	14.29 ± 21.34	12.77 ± 6.28

Note: CG: control group; MCE: motor control exercise; VR: VR training.

participants, but there were no statistical differences in VAS scores among the three groups. The improvement in dysfunction revealed by ODI scores was also not significantly different after training among the three groups. Virtual walking was reported to help reduce pain and kinesiophobia and improve function in the short term in patients with chronic nonspecific LBP [40]. The potential mechanism underlying these findings is that the immediate multisensory feedback provided by VR training can improve pain processing in the CNS [41, 42]. The findings in the MCE group are supported by the results of previous studies [36, 43, 44], which showed that MCE could reduce pain and improve dysfunction in CNLBP patients [45], but the clinical improvements did not statistically differ from other treatments [36, 43, 44]. The potential reason for no between-group differences in the pain-related clinical outcomes of the present study was short-term intervention, which may be not long enough to elicit differential treatment effect. The duration of MCE intervention period of 12 weeks was reported to induce superior effect than general exercise program [46]. However, for the duration of the MCE program of 6 weeks, no significant difference was reported between MCE and generally exercise program [47]. Thus, no firm conclusion could be drawn on the comparison of the effectiveness of each intervention program on pain-related clinical outcomes. VR-based intervention may potentially be a beneficial adjunct to MCE intervention for LBP rehabilitation. Further investigation on VR-based training is at least warranted.

4.1. Limitations. There are several limitations to the present study. First, sample size was calculated only based on the core muscle of TrA rather than other muscles, which may reduce the statistical power of other muscles. The present study was a preliminary study to explore the effect of VR training on postural control. Large trial is required in the future study. Second, the age range of the sample population was only 19–30 years. Thus, the results may not be directly generalizable to participants beyond this age range. Third, the intervention period was only two weeks, which was potentially not long enough to elicit the treatment effect. Future studies should lengthen the training period to six or more weeks to further confirm the effect of VR training on APAs and pain-related clinical outcomes. In addition to assessments of behavioral outcomes, brain imaging and electrophysiological techniques could be employed to investigate the underlying neural mechanisms in future research.

4.2. Conclusions. VR-based training may be an alternative to MCE to enhance APAs by altering the muscle activation pattern of the trunk and lower limb muscles in response to perturbation. The results of this study provide a new potential treatment for APA impairment in CLBP. However, the effect of VR training on the clinical pain symptoms requires further work to verify. In addition to sEMG, future studies can use electrophysiological and brain imaging methods to investigate the underlying neural mechanisms for the effects of VR training on postural control.

Data Availability

The EMG datasets analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declared no conflict of interest.

Authors' Contributions

Zhicheng Li and Qiuhua Yu contributed equally to this work.

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Review Article

Global Research on Neuropathic Pain Rehabilitation over the Last 20 Years

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Background. Neuropathic pain has long been a very popular and productive field of clinical research. Neuropathic pain is difficult to cure radically because of its complicated etiology and uncertain pathogenesis. As pain worsens and persists, pain recovery techniques become more important, and medication alone is insufficient. No summary of bibliometric studies on neuropathic pain rehabilitation is yet available. The purpose of the present study is to analyze in a systematic manner the trends of neuropathic pain rehabilitation research over the period of 2000–2019. *Methods*. Studies related to neuropathic pain rehabilitation and published between January 2000 and December 2019 were obtained from the Science Citation Index-Expanded of Web of Science. No restrictions on language, literature type, or species were established. CiteSpace V and Microsoft Excel were used to capture basic information and highlights in the field. *Results*. Linear regression analysis showed that the number of publications on neuropathic pain rehabilitation significantly increased over time ($P < 0.001$). The United States showed absolute strength in terms of number of papers published, influence, and cooperation with other countries. Based on the subject categories of the Web of Science, "Rehabilitation" had the highest number of published papers (446), the highest number of citations (10,954), and the highest number of open-access papers (151); moreover, this category and "Clinical Neurology" had the same H-index (i.e., 52). "Randomized Controlled Trials" revealed the largest cluster in the cocitation map of references. The latest burst keywords included "Exercise" (2014–2019), "Functional Recovery" (2015–2019), and "Questionnaire" (2015–2019). *Conclusion*. This study provides valuable information for neuropathic pain rehabilitation researchers seeking fresh viewpoints related to collaborators, cooperative institutions, and popular topics in this field. Some new research trends are also highlighted.

1. Introduction

Neuropathic pain is a very popular and productive field of clinical research. In 2008, the IASP Special Interest Group (NeuPSIG) updated its definition of neuropathic pain as pain caused by a lesion or disease of the somatosensory system. Neuropathic pain is a fairly common disorder. Indeed, *Pain* reported that the best estimate of the prevalence of pain with neuropathic characteristics in the population may be between 6.9 and 10% [1–4]. Neuropathic pain is difficult to cure radically because of its complicated etiology and uncertain pathogenesis. This disease not only affects the quality of life and functions of patients but also increases the incidence of depression and anxiety, resulting in the wastage of medical resources and massive economic burdens [5, 6]. As pain worsens and persists, pain recovery techniques become more important, and medication alone is insufficient [7]. Psychosocial support and cognitive behavioral therapy may also be considered. Neuromodulation technology, minimally invasive technology, kinesiotherapy, traditional regimen, and multimodal management plans have shown good effects on pain management [8–14]. The rehabilitation of neuropathic pain is of great significance in addressing the symptoms and improving the clinical prognosis of patients [15].

No summary of the existing research on neuropathic pain rehabilitation is yet available. Bibliometrics combines mathematics, statistics, and philology to conduct quantitative research and analysis on a certain interdisciplinary field. It

is an important academic link to obtain quantifiable, reproducible, and objective data [16]. In addition, bibliometrics can be used as a search tool to analyze the scope of impact of research findings and identify links between relevant and updated research, author networks, and institutions [17]. The Web of Science (WoS) is an online database of scientific citations that can be used to obtain data on citations, subjects, authors, institutions, and impact factors, thereby providing a useful search-and-analysis tool to generate representative data. The CiteSpace can be used to process and export search results directly to analyze published papers. Several articles on cancer rehabilitation, spinal cord injury (SCI) rehabilitation, traumatic brain injury rehabilitation, and total knee arthroplasty rehabilitation have been published [18–21]. This article mainly focused on the rehabilitation of neuropathic pain.

This review analyzes the current publications and development trends of neuropathic pain rehabilitation from the perspective of bibliometrics. The main institutions, extent of international cooperation, current situation, and trends are analyzed, and keyword cluster and world map analyses are used to reveal the research hot spots and leading countries in this field. A detailed bibliometric analysis of neuropathic pain rehabilitation research may help clinicians quickly and accurately classify and understand this field and guide future research directions.

2. Data and Methods

2.1. Data Collection. We collected synonyms related to neuropathic pain and rehabilitation and used "Subject Terms" for retrieval. The screening and downloading of literature for analysis was conducted on November 21, 2020. Literature from the last two decades (years 2000–2019) was downloaded from the Science Citation Index Expanded (SCI-Expanded) database of WoS.

Our search strategy was as follows: TS= (neuralgia∗ OR neurodynia∗ OR sciatica OR "nerve pain∗" OR "nerve cut" OR "nerve constriction" OR "nerve inflammation" OR "nerve crush" OR "nerve injury" OR "nerve ligation" OR "neuropathic pain" OR "peripheral neuropathy" OR "diabetic neuropathy" OR "chronic constriction injury") AND TS= ("rehabilitation" or "physical medicine" or "physical therap∗" or "occupational therap∗").

All of the data in this paper were extracted independently by the author (Xuan Su). EndNote X8 (Bld 7072, Thomson Research Soft, Stamford, CA, USA) and Microsoft Office Excel were used to extract the data to be downloaded from WoS. We strictly followed the established retrieval strategy, extracted the target literature collection, created the citation report, and then obtained the target data. Data on publication count, citation frequency (including self-citations), number of citations per year, number of citations in 2019, H-index, open-access papers, and essential science indicator (ESI) top papers were directly obtained data from WOS and used as bibliometric indicators for visual analysis. All of the relevant data and references were stored in text format for subsequent visualization analysis. Publication count refers to the quantitative contribution of an author or institution. The number of citations, which refers to the sum of citations of all items in a set, can indicate the average quality of published papers. The H-index, also known as the H-factor, was proposed by Hirsch. This index evaluates authors' academic achievements in a specific field [22, 23]. For instance, if the H-index of an author is 30, all papers published by the author have been cited at least 30 times in 30 papers. Higher H-index values indicate more influential and persuasive papers. Open-access papers refer to the number of publications whose peer-reviewed versions are available free of charge from a publisher's website or repository.

2.2. Inclusion Criteria. In this study, papers published in a wide variety of periodicals, including *Pain*, *Lancet*, and *Brain*, on neuropathic pain rehabilitation were included without restrictions on the type of article or language used. The types of literature mainly included articles, reviews, and proceedings. Both animal and clinical studies were included.

2.3. Statistical Analysis. The data were imported into CiteSpace (5.3.R11) in plain text format for analysis. CiteSpace V and Microsoft Excel were used to capture basic information and notable points in the field. The characteristics of the field were then studied in terms of discipline terms and keywords, and the publishing model of papers was assessed in terms of the number of publications in each country and the journal publishers. The frequency and percentage of journal and annual publications in each country were calculated on the basis of year of publication. The variation trends of research hot spots were studied through citation frequencies, keywords, and timeline views. Finally, we analyzed the citation trends of the top 10 countries, top 10 journals, and top 10 research fields to explore publishing patterns. IBM SPSS Statistics 22.0 (SPSS, Inc., Chicago, IL, USA) was used to calculate the number of changes and determine whether the data are statistically significant. Linear regression analysis was performed on the data using category as the dependent variable and year as the independent variable. For example, analyze the number of articles published each year. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Publication Outputs and Growth Trend. A total of 1,518 papers conforming with the retrieval requirements were collected. Articles and reviews accounted for 94.1% of the total number of articles collected. The remaining literature types included non-article-type documents, including proceedings papers, editorial materials, meeting abstracts, book chapters, early-access articles, corrections, letters, and reprints.

The annual publication volume generally increased with some fluctuations over the years (Figure 1). A statistically significant increase in number of papers published, from 21 articles in 2000 to 140 articles in 2019, was noted ($t = 16.795$, $P < 0.001$), thereby indicating that medical researchers are gradually expanding the field of research on the rehabilitation of neuropathic pain.

From 2000 to 2019, 1,518 papers were published in the field of neuropathic pain rehabilitation (average, 75 papers

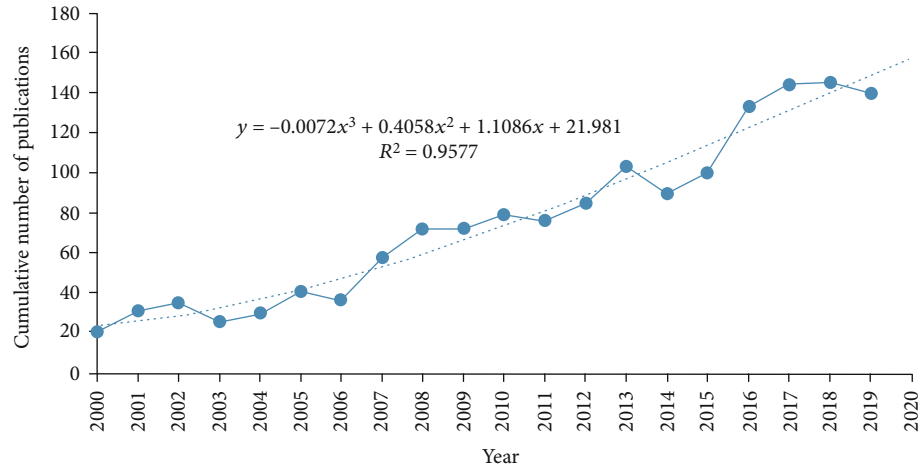

Figure 1: The number of annual publications on neuropathic pain rehabilitation research from 2000 to2019 and establish a time trend citation curve.

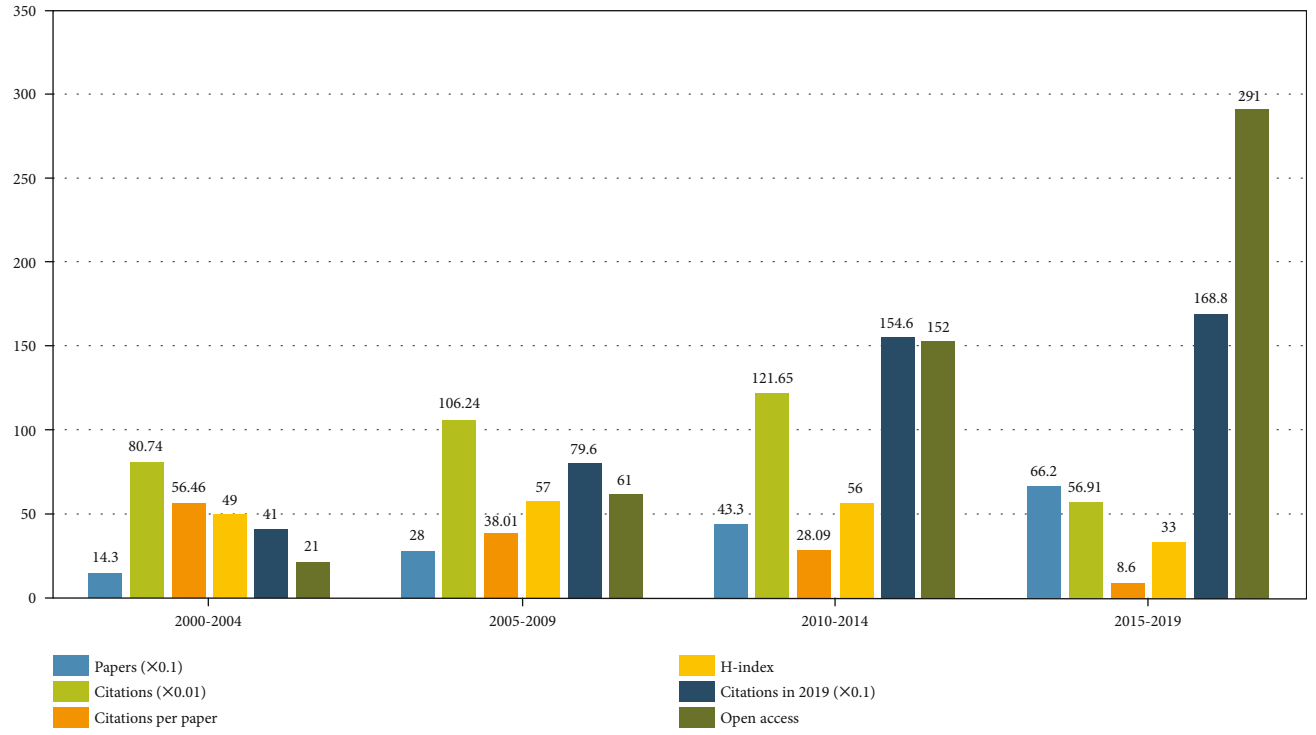

Figure 2: Number of papers, citations, citations per paper, open access paper, H-index, and citations in 2019 for each 5-year time period.

per year). The number of publications published in 2020 was forecasted on the basis of the growth rate curve of the number of publications by using the formular growth rate $= -0.0072x^3 + 0.4058x^2 + 1.1086x + 21.981$ $(R^2 = 0.9577)$, and the predicted number of papers to be published in 2020 was 158.

Among the four 5-year periods established (2000–2004, 2005–2009, 2010–2014, and 2015–2019), the most cited period per paper (5,646 times) was 2000–2004 (Figure 2), likely because this period represents the early stage of rehabilitation professional development, and only a small number of articles were available at the time. Although the number of articles published in this period was only 143, the total number of citations (8,074 times) in this period exceeded that in 2015–2019 (5,691 times). The H-index peaked from 2010 to 2014. The citations in 2019 and the number of open-access articles peaked from 2015 to 2019.

3.2. Distribution of National Geography and Institutions. Figure 3 shows a world map of all countries and territories in which studies on neuropathic pain rehabilitation had been published; here, the geographical distribution of publications covered 63 countries and territories. Figure 4(a) shows the extensive cooperation between countries and regions.

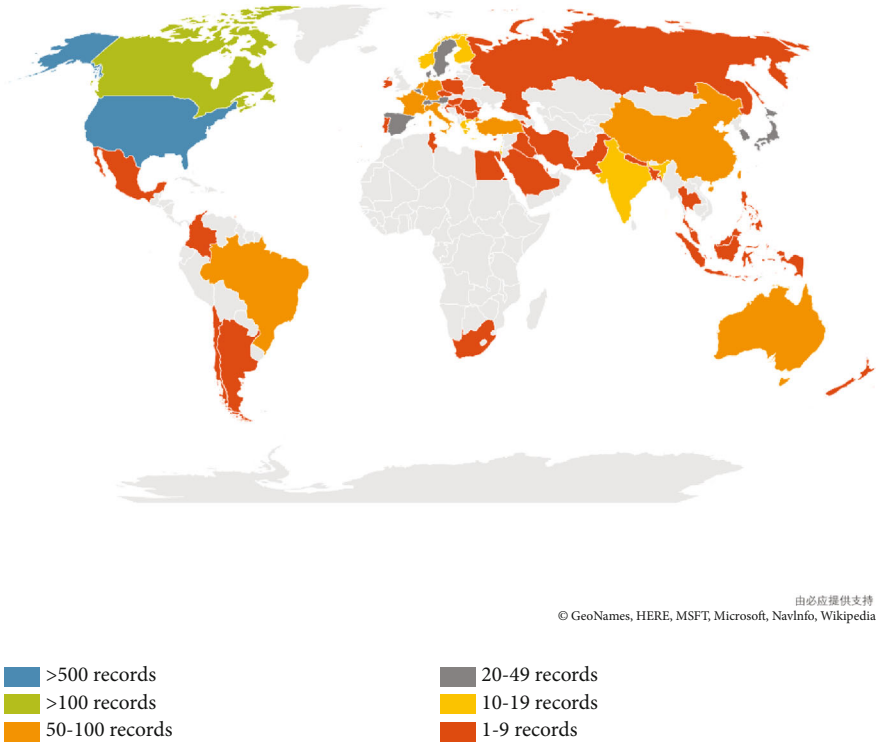

Figure 3: World map of total country output based on neuropathic pain rehabilitation research.

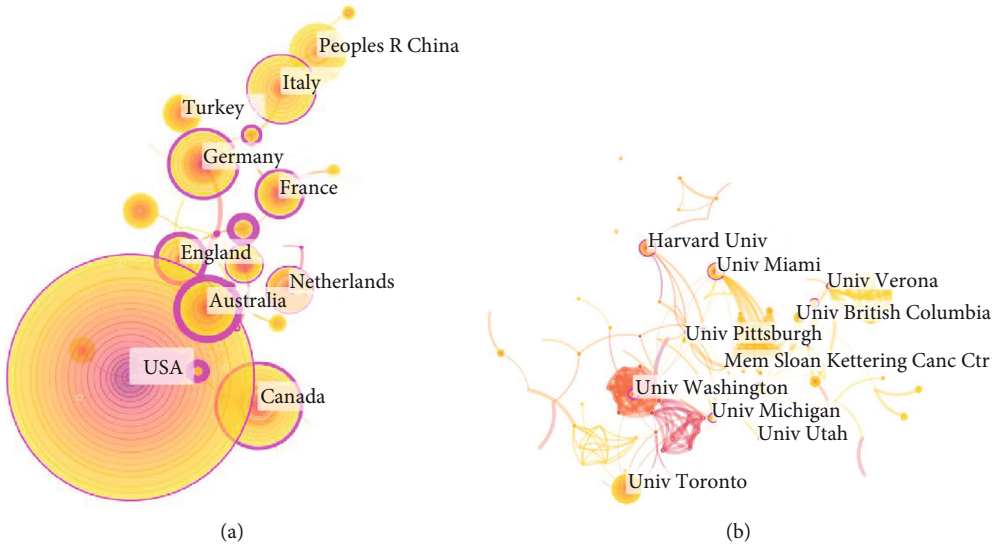

Figure 4: The analysis of countries and institutions. (a) Network map of countries/territories engaged in neuropathic pain rehabilitation research. (b) Network map of institutions engaged in neuropathic pain rehabilitation research.

Table 1 reveals the top 10 countries by number of published papers. The United States demonstrated a strong influence in this field, publishing the largest number of papers and five ESI top papers. Studies published in the United States also showed the largest number of citations (19,543 times), the highest H-index (71), and the greatest number of open-access articles (196).

Published papers in the field of neuropathic pain rehabilitation involved a total of 2,014 institutions. Table 2 shows the top 10 institutions in terms of number of papers published. The papers of the Harvard University were cited the most (2,278 times), but the papers of the Mayo Clinic, the no. 1 hospital in the United States, were cited the most citations per year (112.85 times), with two ESI papers. The University of Toronto had the highest number of published papers (36), the University of Washington had the highest H-index (21), and the University of Pittsburgh had the highest number of open-access articles (15). Figure 4(b) shows the

Table 1: The top 10 countries of origin of papers in neuropathic pain rehabilitation research.

Country	Papers	Citations WoS	Citations per paper	Open access	*H*-index	ESI top paper
USA	599	19543	32.63	196	71	5
Canada	113	2867	25.37	44	30	0
Peoples R China	92	843	9.16	50	16	0
Germany	91	2134	23.45	20	24	0
Italy	85	2919	34.34	22	25	1
Austria	76	4414	58.08	32	26	1
Netherlands	67	2345	35	37	28	0
England	65	2672	41.11	32	22	1
France	61	2376	38.95	12	19	1
Turkey	60	569	9.48	16	12	0

Table 2: The top 10 institutions of origin of papers in neuropathic pain rehabilitation research.

Institutions	Papers	Citations WoS	Citations per paper	Open access	*H*-index	ESI top paper
Univ Toronto	36	980	27.22	12	17	0
Harvard Univ	31	2278	73.48	12	19	1
Univ Washington	27	2042	75.63	10	21	0
Univ Miami	26	698	26.85	14	12	0
Univ Michigan	24	997	41.54	8	14	1
Univ Pittsburgh	22	480	21.82	15	11	0
Washington Univ	22	1086	49.36	11	14	0
Mayo Clin	20	2257	112.85	8	15	2
Mem Sloan Ketteing Canc CTR	19	1098	57.79	7	15	1
Univ British Columbia	19	723	38.05	12	12	0

Table 3: The top 10 authors, cocited authors, and cocited references in neuropathic pain rehabilitation research.

Author	Count	Cocited author	Count	Cocited reference	Count
Vera Bril	6	Finnerup NB	113	Attal N, 2010, Eur J Neurol, V17, P1113	22
Run Wang	6	Siddall PJ	95	Kirshblum SC, 2011, J Spinal Cord Med, V34, P547	21
Stefano Tamburin	6	Dworkin RH	90	Treede RD, 2008, Neurology, V70, P1630	21
Julie M Fritz	6	Jensen MP	80	Finnerup NB, 2015, Lancet Neurol, V14, P162	20
J J Labat	5	Woolf CJ	64	Haanpaa M, 2011, Pain, V152, P14	20
R Robert	5	Attal N	63	Woolf CJ, 2011, Pain, V152, P0	20
N B FInnerup	5	Bouhassira D	60	Dworkin RH, 2007, Pain, V132, P237	14
T Riant	5	Melzack R	58	Mulhall JP, 2008, J Sex Med, V5, P1126	14
B C Craven	4	Baron R	50	Backonja M, 1998, Jama-J AM MED Assoc, V280, P1831	12
A Townson	4	Harden RN	48	Van de Vusse AC, 2004, BMC Neurol, V4, P0	12

degree of cooperation among the top 10 institutions engaged in neuropathic pain rehabilitation research. According to our analysis of countries and institutions, the Harvard University is the world's leading university in this field and the center of a cooperative network.

3.3. Analysis of the Top 10 Authors and Cocited Authors. A total of 1,518 papers on neuropathic pain rehabilitation research were written by 6,180 authors. Among the top 10 cocited authors (Table 3), Finnerup NB was cited 113 times, followed by Siddall PJ (95 times), and Dworkin RH (90 times). These authors are active and influential in the field of neuropathic pain rehabilitation. Vera Bril, from the Division of Neurology, Toronto General Hospital in Canada, studied the occurrence and development of diabetes and performed research on various possible neuropathies, including the clinical manifestations, diagnostic characteristics, and management of various sequelae [24–27]. Stefano Tamburin from the Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, found that the use of psychotherapy has a pain-relieving effect on neurological disorders. The author's team also demonstrated that

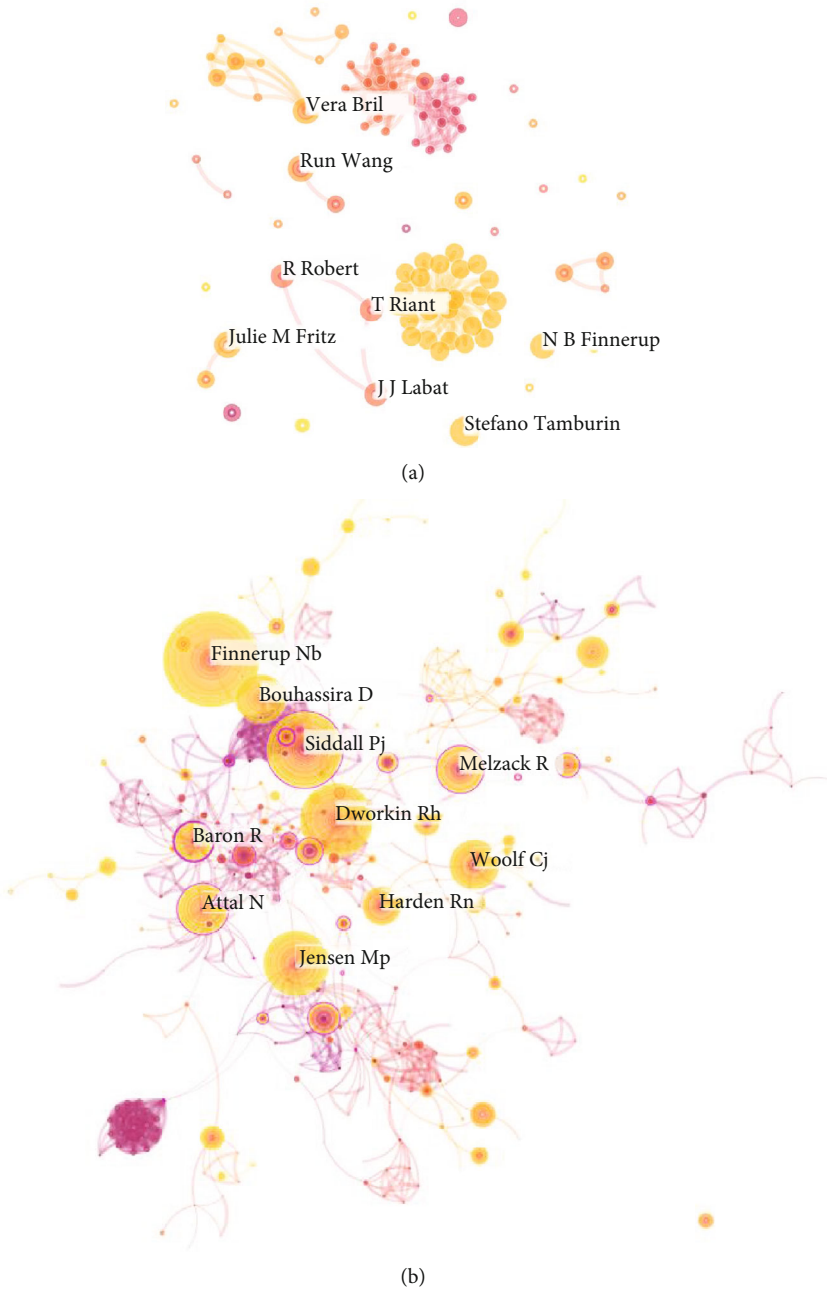

Figure 5: The analysis of authors. (a) Network map of active authors contributed to neuropathic pain rehabilitation research. (b) Network map of cocited authors contributed to neuropathic pain rehabilitation research.

different forms of psychological intervention measures, including cognitive behavior therapy, hypnosis, cognitive or behavioral techniques, mindfulness, acceptance and commitment therapy, brief interpersonal therapy, and virtual reality interventions, could effectively reduce the morbidity of different pains, such as musculoskeletal pain, fibromyalgia, central poststroke pain, phantom limb pain, pain secondary to SCI, diabetic neuropathy migraines and headaches, complex regional pain syndrome (CRPS), and medically unexplained symptoms [28–32]. In other words, pain is inextricably linked to cognition.

Figure 5 illustrates author and cocited author cooperation maps. These two graphs provide effective and intuitive information that allows readers to observe the collaboration between authors. However, the centrality of cooperation at the author level is generally less than 0.03, thereby indicating that cooperation between researchers is not so close with certain limitations.

3.4. Analysis of the Top 10 Cocited References. References are an important component of high-quality papers that not only provides a strong argument for the author's findings but also

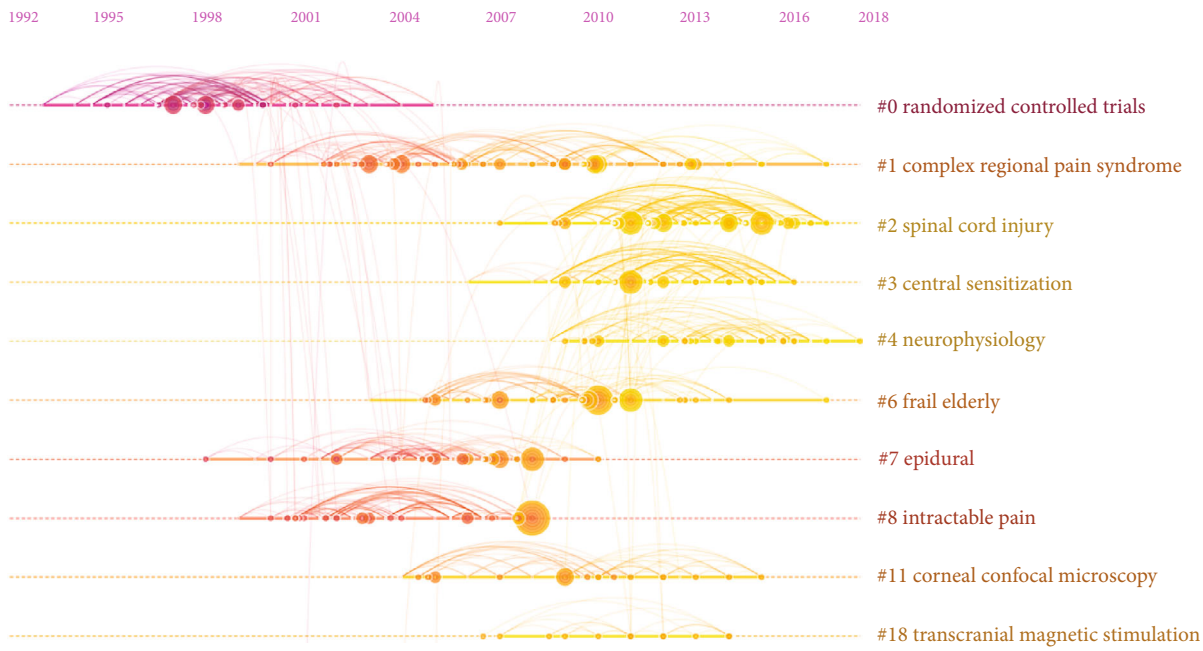

Figure 6: The analysis of references. Cocitation map (timeline view) of references from publications on neuropathic pain rehabilitation research.

Table 4: The top 10 journal of origin of papers in the neuropathic pain rehabilitation research.

Journals	Papers	Citations WoS	Citations per paper	WoS categories	IF 2019	Quartile	*H* -index
Archives of Physical Medicine and Rehabilitation	128	4031	31.49	Rehabilitation; sport sciences	3.098	Q1; Q1	37
Spinal Cord	34	1024	30.12	Clinical neurology; rehabilitation	1.773	Q3; Q2	14
American Journal of Physical Medicine Rehabilitation	27	593	21.96	Rehabilitation; sport sciences	1.838	Q2; Q3	10
European Journal of Physical and Rehabilitation Medicine	23	240	10.43	Rehabilitation	2.258	Q1	9
Physical Therapy	23	1823	79.26	Orthopedics; rehabilitation	3.14	Q1; Q1	13
Journal of Rehabilitation Research and Development	20	554	27.7	Rehabilitation (SSCI); rehabilitation (SCIE)	1.277 (2016)	Q2; Q3	16
Neurorehabilitation and Neural Repair	18	614	34.11	Clinical neurology; rehabilitation	3.982	Q1; Q1	10
PM R	18	347	19.28	Rehabilitation; sport sciences	1.821	Q2; Q3	8
Journal of Sexual Medicine	16	505	31.56	Urology and nephrology	3.293	Q2	10
Pain Medicine	16	135	8.44	Anesthesiology; medicine, general and internal	2.513	Q2; Q2	8

expands the information chain and reflects the scientific value of research. In other words, references are an important index that reflects the scientific basis of a paper.

A timeline view of the literature cocitation analysis is shown in Figure 6; here, active clusters named after the index terms cited in the literature are listed. Modularity, which is expressed as *Q* value, is a commonly used method to evaluate the strength of the network community structure. In this study, the *Q* value was 0.8705. A *Q* value higher than 0.3 indicates that the community structure is significant. The largest cluster (#0) was "randomized controlled trials," followed by "complex regional pain syndrome" (#1), "spinal cord injury" (#2), and "central sensitization" (#3). Despite the fairly wide availability of research on the mechanisms involved in central sensitization or neuroinflammation in patients with chronic low back pain or musculoskeletal pain, the treatment of these issues remains a challenging scientific problem [33–37]. Experts recommend pain neuroscience education, cognitive behavioral therapy, and exercise therapy [38].

3.5. Bibliometric Analysis of the Journals. Over the last 20 years (2000–2019), a total of 585 journals published papers

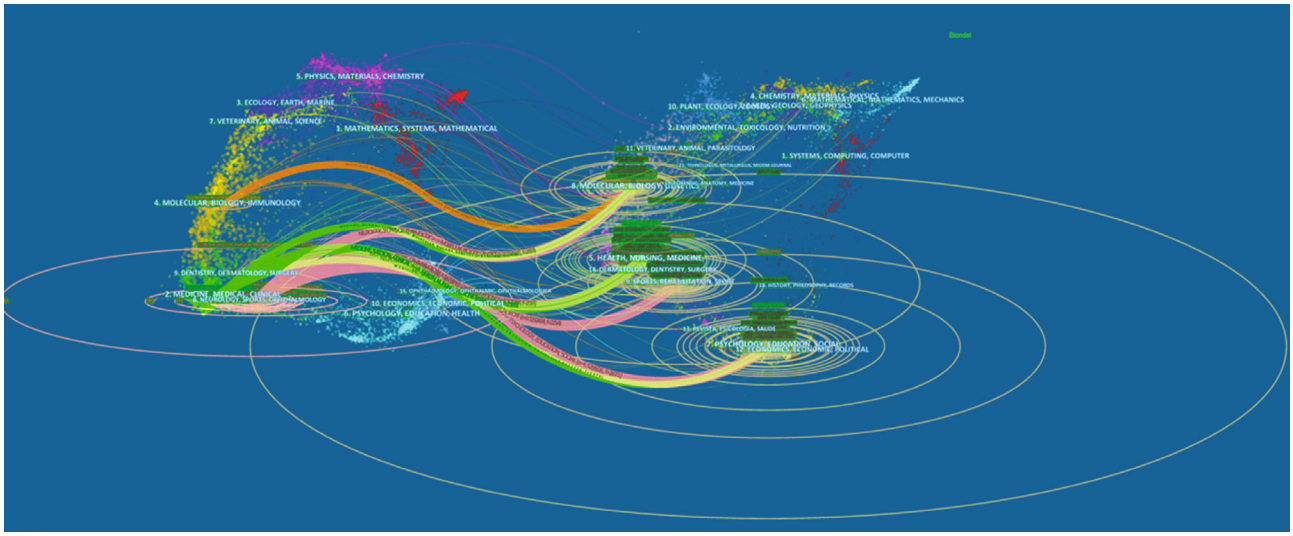

Figure 7: The dual-map overlay of journals related to on neuropathic pain rehabilitation research.

related to neuropathic pain rehabilitation. Active journals are defined as journals with the greatest number of publications within a certain period. As shown in Table 4, the top 10 active journals published 323 articles, accounting for 21.28% of the total number of published papers. Most journals have an impact factor ranging from 2 to 4. The journals with the highest and lowest impact factors were *Neurorehabilitation and Neural Repair* (IF 2019, 3.982) and *Journal of Rehabilitation Research and Development* (IF 2016, 1.277), respectively. Because of the diversity of categories, four journals remained in Q1, thereby indicating that the overall impact factor of published papers in this field is not high. However, some categories such as "rehabilitation" and "exercise science" could be found in Q1.

A dual-map overlay of journals is shown in Figure 7. The figure shows the disciplines covered by the journal in the form of labels. The colored line segment in the dual-map represents the cited connections, and the connection traces the citing journal to the cited journal. The number of authors is shown on the horizontal axis of the ellipse, whereas the number of publications is shown on the vertical axis of the ellipse. Most of the papers were published in journals dedicated to neurology, sports, and ophthalmology (left), and these journals were mostly cited by journals of psychology, education, and society (right).

3.6. Distribution of Keywords. Keywords are the core summary of a paper, which can be analyzed on the topic of the paper. Authors generally believe that the more frequently lexical pairs appear in the same literature, the closer the relationship between these two topics. The most common keywords were diabetes mellitus, neuropathic pain, low back pain, and spinal cord injury (as shown in Figure 8).

The frequent occurrence of keywords over a certain period of time is regarded as an indicator of frontier topics, vigorous development, and emerging trends. Keywords with the strongest citation bursts were derived from CiteSpace, and the results are shown in Figure 9. Here, the top 25 highlighted keywords from 2000 to 2019 are highlighted.

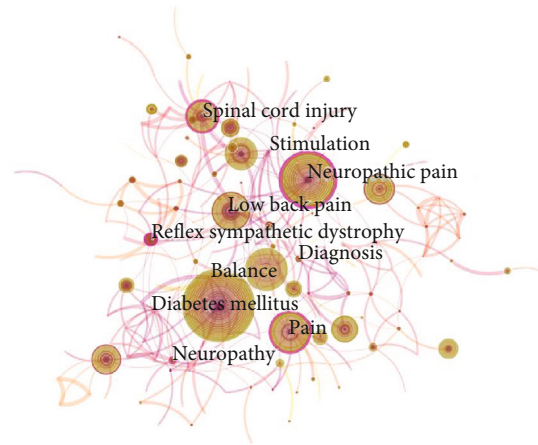

Figure 8: Network map of keyword cooccurrence in neuropathic pain rehabilitation research from 2000 to 2019.

Exercise, functional recovery, outcome, and questionnaire appeared to be newly developing research hot spots. The keyword "reflex sympathetic dystrophy," which appeared only in 2012 had the highest citation burst rate (8.99).

3.7. Analysis of the Subject Categories. The 1,518 papers on neuropathic pain rehabilitation research were allocated to 87 topic categories in WoS. Among the top 20 subject categories (Figure 10), "*Rehabilitation*" revealed the highest number of published papers (446), citations (10,954), and open-access papers (151). "*Anesthesiology*" showed the highest number of citations per item (26.64), and *Engineering Biomedical* demonstrated the highest number of citations per paper (65.42). The *H*-index of *Rehabilitation* and *Clinical Neurology* was identical at 52.

Linear regression analysis showed that the percentages statistically increased over time ($P < 0.001$) in the top 20 categories (*Rehabilitation*, *Clinical Neurology*, *Sport Sciences*, *Orthopedics*, *Neurosciences*, *Surgery*, *Anesthesiology*, *Medicine General Internal*, *Urology nephrology*, *Oncology*,

Top 25 keywords with the strongest citation bursts

Keywords	Year	Strength	Begin	End	2000 - 2019
Reflex sympathetic dystrophy	2000	8.999	2000	2012	
Complex regional pain syndrome	2000	3.7937	2000	2011	
Gabapentin	2000	6.2616	2000	2003	
Diabetic neuropathy	2000	7.2278	2000	2007	
Neuralgia	2000	3.7059	2000	2007	
Muscle	2000	3.0855	2001	2004	
Back pain	2000	5.0233	2002	2008	
Therapy	2000	5.6297	2002	2005	
Postherpetic neuralgia	2000	4.0039	2002	2011	
Controlled trial	2000	4.3268	2005	2012	
Injection	2000	3.7397	2005	2008	
Neuropathy	2000	3.7391	2006	2009	
Efficacy	2000	4.0865	2007	2010	
Syndrome type i	2000	6.1593	2008	2014	
Dysfunction	2000	5.1568	2009	2013	
Follow up	2000	3.9859	2011	2014	
Spasticity	2000	3.1839	2011	2015	
Prevalence	2000	5.2877	2013	2016	
People	2000	8.3532	2013	2016	
Chronic pain	2000	7.2719	2013	2016	
Exercise	2000	5.6256	2014	2019	
Complication	2000	4.9433	2014	2017	
Functional recovery	2000	3.8605	2015	2019	
Outcm	2000	7.502	2015	2019	
Questionnaire	2000	4.4582	2015	2019	

Figure 9: The keywords with the strongest citation bursts of publications on neuropathic pain rehabilitation research.

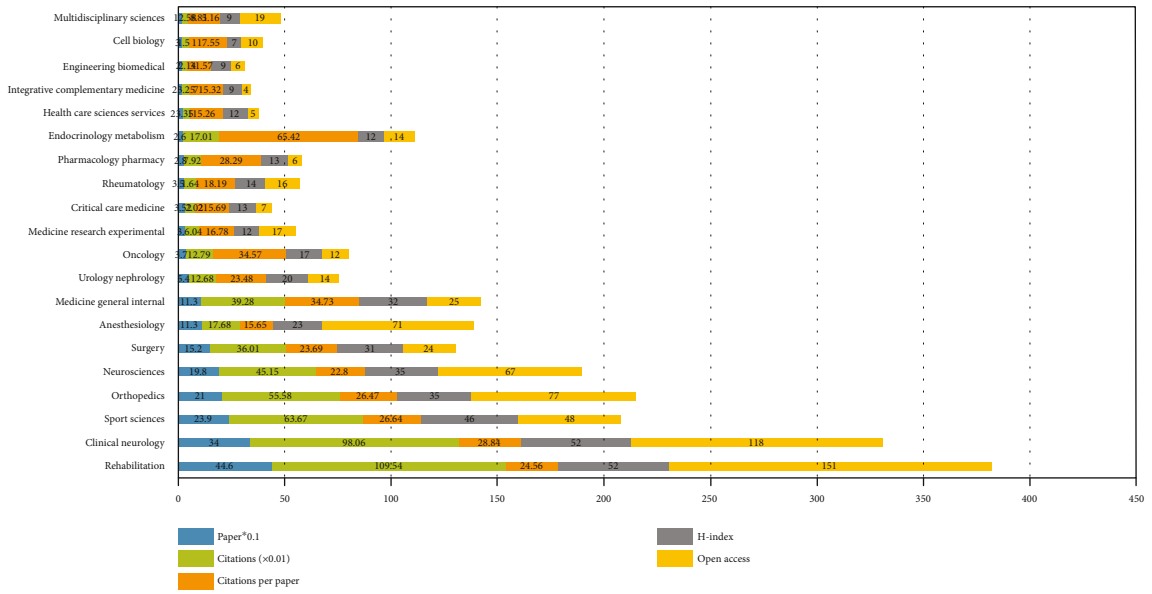

Figure 10: The number of papers, citations, citations per paper, open access papers, and *H*-index of the top 20 subject categories of the Web of Science.

Medicine Research Experimental, *Critical Care Medicine*, *Rheumatology*, *Pharmacology Pharmacy*, *Endocrinology Metabolism*, *Health Care Science Services*, *Integrative Complementary*, *Engineering Biomedical*, *Cell Biology*, and *Multidisciplinary Sciences*).

4. Discussion

4.1. Global Tendency of Neuropathic Pain Rehabilitation Research. In this review, the CiteSpace V software was used to carry out bibliometrics analysis in the field of neuropathic

pain rehabilitation. Changes in bibliometric indicators, such as keywords, subject words, authors, countries, and institutions, over a time span of 20 years were then presented in diagrams and tables.

The output of publications showed a gradual annual increase (Figure 1). According to the number of papers published in the field of neuropathic pain rehabilitation in different countries as well as the overview of countries on the world map, the United States was relatively productive in this field of research (599), followed by Canada (113), China (92), and Germany (91). The top 10 countries/regions included five European countries (i.e., Germany, Italy, Netherlands, England, and France), two Asian countries (i.e., China and Turkey), two North American countries (i.e., the USA and Canada), and one Oceanic country (i.e., Australia). Figure 4(a) shows that several countries, especially European Union countries, are closely linked together. A total of 2,014 institutions contributed publications on neuropathic pain rehabilitation research. Nine of the top 10 institutions were found in the United States and one is in Canada. Two non-university institutions, namely, the Mayo Clinic and Memorial Sloan Kettering Cancer Center, are in the top 10 institutions and published two ESI Top papers and one ESI Top paper, respectively. The United States, as a developed country, is clearly the leader in this field.

4.2. Research Hot Spots and Trends. As an emerging field, rehabilitation medicine has received extensive attention in recent years on account of its important role in SCI, cerebral apoplexy, and osteoarthropathy. We explored emerging topics and concerns in the field of neuropathic pain rehabilitation.

Analysis of the comorbidity map of the references showed that "randomized controlled trials" was the largest cluster, and the other large clusters are as follows:

(1) Complex regional pain syndrome: CPRS presents as a type of burning pain and is usually caused by neuropathic pain. Its pathogenesis involves neurogenic inflammation mediated by cytokines and neuropeptides. Studies have shown that spinal cord stimulation, dorsal root ganglion stimulation (DRGS), is effective in treating the disease [39, 40]

(2) Spinal cord injury: chronic neuropathic pain after SCI is a complex disease, and transcranial direct current stimulation is effective in clinical treatment [41]. The latest clinical practice guidelines also point to the use of sensors and mechanical devices can help patients achieve functional movement, enhance recovery, and increase neural plasticity, as well as potential adjuncts [42–44]

(3) Central sensitization: central sensitization is a kind of hypersensitivity to pain caused by central neural plasticity, which is interwoven with psychoneuroimmunological interactions [34, 35], and is of great significance for the diagnosis and treatment of pain. Recent studies have shown that cGMP-dependent protein kinase I, a nociceptor locator, is a key producer of central sensitization and neuropathic pain [45]. We found that activation of microglia attenuated synaptic transmission and reduced neuroinflammation, synaptic function, and neuralgia. Therefore, chemotherapy offers a potential opportunity to explore microglia function and neuropathic pain treatment [46]

Analysis of the keywords with the strongest citation bursts from 2000 to 2019 revealed major hot spots in the field of neuropathic pain rehabilitation (as shown in Figure 6). The top 25 keywords in 2000 included "reflex sympathetic dystrophy," "complex regional pain syndrome," "gabapentin," "diabetic neuropathy," and "neuralgia." The top 25 keywords by the end of 2019 included "exercise" (2014–2019), "functional recovery" (2015–2019), "outcome" (2015–2018), and "questionnaire" (2015–2018). These keywords may predict the frontiers of research as follows:

(1) Exercise

In recent years, the idea that exercise is good medicine has been widely accepted by the public, and exercise is among the methods recommended for the treatment of neuropathic pain [47]. Although the mechanism of exercise in improving neuropathic pain has been confirmed in animal experiments, the corresponding mechanism in humans is complex and has not been thoroughly studied.

The effect of sports on the improvement of lower back pain, diabetic neuralgia, and pediatric pain has also been affirmed by professionals [48–50]. Exercise therapy can help patients avoid the adverse effects of drug therapy, relieve pain, and improve their quality of life.

(2) Functional recovery

No evidence from randomized trials indicates that treatment is necessarily effective. For example, randomized clinical trials are needed to determine the efficacy of glucocorticoids or other immunoregulatory therapies in the treatment of neuralgia muscular atrophy [51]. In one experiment, long-term regular exercise was explored as a means to reduce the neuroanalgesic behavior of mice and, ultimately, promote motor function [52]. Another study investigated the efficacy and functional recovery of SCI neuropathic pain symptoms by using long-term intensive locomotor training [53].

(3) Outcome

The efficacy of different interventions in the treatment of neuropathic pain could be evaluated by analyzing data on pain, function, dose, and adverse effects in randomized controlled trials. Knowledge of outcomes can help patients choose the appropriate rehabilitation treatment [54, 55].

4.3. Strength and Limitations. This article is the first to summarize the research current status, geographical distribution, research hot spots, and development trends in neuropathic pain rehabilitation worldwide. Our study encompasses 20

years of data extracted from WoS and analyzed by CiteSpace and, thus, provides strong evidence of the future development of research in this field through keywords and subject categories. The soft power of science and technology of each country was visualized using a world map of the distribution of published papers, institutions, journals, and countries. Analysis of the authors and cited authors could help identify leaders in this domain. However, the limitations of our work must be acknowledged. First, although we believe that WoS is a suitably large database that can provide a wide variety of publications critical to our analysis, future researchers could use other databases, such as Scopus, Embase, Ovid-Medline, and China Knowledge Resource Integrated (CNKI), to explore other potential papers. Future studies can broaden the search scope to include more relevant studies to enrich the literature. Finally, some keywords that did not provide much information, such as risk, model, and system, could not be analyzed.

5. Conclusion

Our understanding of neuropathic pain rehabilitation has advanced remarkably over the last 20 years. Using bibliometric charts, we illustrated the overall structure of scientific research on neuropathic pain rehabilitation and provided comprehensive information related to this field for other investigators. The most recent burst keywords were "exercise," "functional recovery," "outcome," and "questionnaire." This analysis provides a comprehensive overview of relevant research conducted in the area of neuropathic pain rehabilitation.

Data Availability

All research data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors report no conflicts of interest.

Acknowledgments

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Research Article

Low Back Pain Assessment Based on Alpha Oscillation Changes in Spontaneous Electroencephalogram (EEG)

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Objectively and accurately assessing pain in clinical settings is challenging. Previous studies showed that alpha oscillations of electroencephalogram data are correlated with subjective perceived pain. Based on this finding, this study is aimed at assessing chronic low back pain based on alpha oscillations. Multichannel electroencephalogram data were recorded from 27 subjects with chronic low back pain under the simple conditions of closing eyes or opening eyes. Spectral analyses were conducted to extract the alpha band responses, and the alpha powers were calculated for the two conditions, respectively. Normalized alpha power was calculated by subtracting the alpha power in the eyes-open condition from that in the eyes-closed condition. The correlation between the alpha power and the subjective pain intensity was evaluated in frontal, central, and posterior regions. The normalized alpha power in the central region was negatively correlated with the subjective pain intensity ($R = -0.50$, $P = 0.01$), with the strongest correlation occurring at the Cz electrode ($R = -0.59$, $P = 0.04$). The correlation analysis results demonstrated the possibility of using the differences of alpha spectral power between eyes-closed and eyes-open conditions as a measure for assessing chronic low back pain. The findings suggest that the normalized alpha power in the central region may be used as a measurable and quantitative indicator of chronic pain for clinical applications.

1. Introduction

Pain, especially chronic pain, is one of the biggest public health problems in our society [1]. A survey by the National Center for Health Statistics found that low back pain (LBP), migraine or severe headache, and joint pain were the most common types of chronic pain in clinical practice [2]. Thus, an effective technique to measure and quantify LBP is needed to achieve better diagnosis and management in clinical settings [3].

Because pain is a subjective individual experience, self-reported pain intensity is considered the gold standard for evaluating pain in clinical situations [1, 2]. However, the clinical pain measure always needs careful evaluation and a skillful clinician to guide [4]. Besides, self-reports of pain intensity are not available for some vulnerable populations, who have severe cognitive or communicative impairments and cannot provide self-reports [5]. Hence, an objective measure of pain intensity that is expected to be associated with standard measures, such as visual analog scale (VAS) [1], can complement self-reports that would be useful in clinical practice, such as to monitor the effect of an analgesic drug or track the recovery of the nociceptive system in non-communicative patients [5, 6].

Electroencephalography (EEG) is a noninvasive monitoring technique that is widely used to probe neurological disorders with high temporal resolution. In recent decades, techniques have been developed to objectively quantify subjective perceived pain through cortical measures derived from EEG responses to brief, phase-locked noxious stimuli [7, 8]. Since brain activity in multiple brain regions elicited by transient painful stimuli is often correlated with pain intensity, researchers are motivated to identify brain activity features that could serve as biomarkers for objective pain assessment [9]. However, such brief stimuli may be limited in their ability to reliably simulate natural and clinically

painful experiences, even when considering acute pain [8]. Therefore, a growing number of studies have focused on developing an objective approach to measuring chronic pain by using sustained pain stimulation, which may evoke non-phase-locked cortical responses similar to those observed in EEG data associated with chronic pain [10–12].

Recording and characterizing cortical electrophysiological responses to chronic pain require measurements other than event-related potentials, namely, continuous EEG. Continuous EEG data are commonly analyzed by transforming them from the time domain to the frequency domain. EEG at different frequency bands including theta, beta, and gamma has been reported to be related to pain perception [13–16], even though there has not been a clear consensus determining which rhythmic band has the most reliable correlation with different levels of elicited pain. Compared with other frequency domains, alpha band oscillations (8–13 Hz) are the most commonly explored [17] (considering that gamma waves are generated deep in the brain and are therefore not easy to record with scalp EEG [18], while data concerning pain-related beta EEG activity are scarce [16, 19, 20]). Furthermore, resting-state EEG recording of alpha oscillations is found to be stable over time [21], highly heritable [22], and unique to an individual to the extent that it could serve as a "statistical signature" [23]. It has been reported that experimentally induced transient pain elicits a decrease in alpha power [9, 11, 21]. Compared with transient noxious painful experiences, findings regarding the effect of tonic pain on alpha oscillatory activity have been inconsistent: some studies have indicated that alpha rhythm induced by tonic pain is suppressed in frontal-central or parietal-occipital regions [15, 24–26], whereas others have reported that alpha oscillations are enhanced over these cortical regions [14, 27]. Although there is no clear consensus regarding how alpha power changes as sustained stimuli are processed, alpha band oscillations are generally considered to be correlated with different levels of pain [18]. Moreover, it has been found that the frequency corresponding to the maximum alpha power at rest predicts an individual's responsiveness to tonic noxious heat stimuli [24].

This previously reported relationship between alpha oscillations and subjective perceived pain encouraged us to investigate whether alpha oscillations obtained from continuous resting EEG are associated with subjective reports of perceived pain in chronic pain patients. However, our previous studies were limited in that the subjects were healthy adults, so tonic noxious stimuli were used to induce pain and elicit the EEG oscillations [15, 26]. Although experimentally induced tonic pain stimuli better resemble the sensory experience in a clinical setting than brief stimuli, they may not involve the same neurological responses as pain [18, 19, 28]. Some electrophysiological and brain imaging studies on patients suffering from chronic pain reported inconsistent findings of the changes in spontaneous oscillatory activity and their association with pain intensity [13, 29–31]. Most of these preliminary studies were conducted in patients suffering from the pain of different origins, and data analyses were not standardized. Therefore, further investigation is needed to expand the previous findings to the pain population. Accordingly, in the present study, we aimed to investigate whether alpha oscillation, an objective neurophysiological parameter, could serve as a measurable and quantitative indicator associated with chronic LBP.

It is well known that alpha activity in EEG data is dominant in normal individuals in the eyes-closed (EC) resting condition and suppressed in the eyes-open (EO) condition [32]. Numerous prior studies have examined the differences between EEG data obtained in the EC and EO conditions in the resting state to determine appropriate baseline readings for protocol development [33]. In general, the EC condition is recommended as a baseline resting state for EEG measurements in various experimental designs, especially those that do not involve tasks with visual stimulation [32]. In addition, Barry and De Blasio [34] reported that, compared with the other bands, the alpha band exhibits a stable widespread reduction in activity from the EC to the EO condition with no topographic changes, allowing the EC and EO data to be pooled across times for subsequent analyses. These findings may imply that compared with the direct recording of EEG oscillations under the EO condition according to a traditional experimental protocol, employing the EC/EO experimental paradigms in which the EC condition is considered a baseline resting state is promising to obtain a relatively stable normalized alpha power. Therefore, we examine possible EEG oscillations in the alpha band (alpha power under the EO condition or normalized alpha power) that is associated with the subjective perceived chronic LBP. We also compare the performance of different parameters for pain assessment, then propose an appropriate one.

2. Materials and Methods

2.1. Subjects. Twenty-seven patients with chronic LBP were recruited in this study, including 20 females and 7 males. Their mean age was 44.6 ± 2.3 (mean $\pm$ SD) years. None of the patients had a history of neurological or psychiatric disease, without previous history of neurological or spinal surgery. They were diagnosed as LBP without specific neuromusculoskeletal pathology, after a careful clinical assessment by experienced clinicians. All subjects have been suffering LBP with an average duration of 5.6 years (range 2–20 years). Subjects to be included in this study were asked to read the study protocol carefully. Those subjects that cannot understand the protocol to follow the guidance of clinicians very well were excluded.

Each subject provided informed written consent before each experiment. The study was conducted according to the guidelines of the Declaration of Helsinki. The experimental protocol was approved by the Institutional Review Board of the University of Hong Kong and West Cluster of Hospital Authority (UW 08-181). The study was registered on the Clinical Trial Registry (https://register.clinicaltrials.gov/) with the registration number NCT03511404.

2.2. EEG Recording. Continuous EEG recording was conducted using a 64-channel Neuroscan System (Compumedics Limited, Victoria, Australia) using an electrode cap according to the 10-20 system. The system was set up with

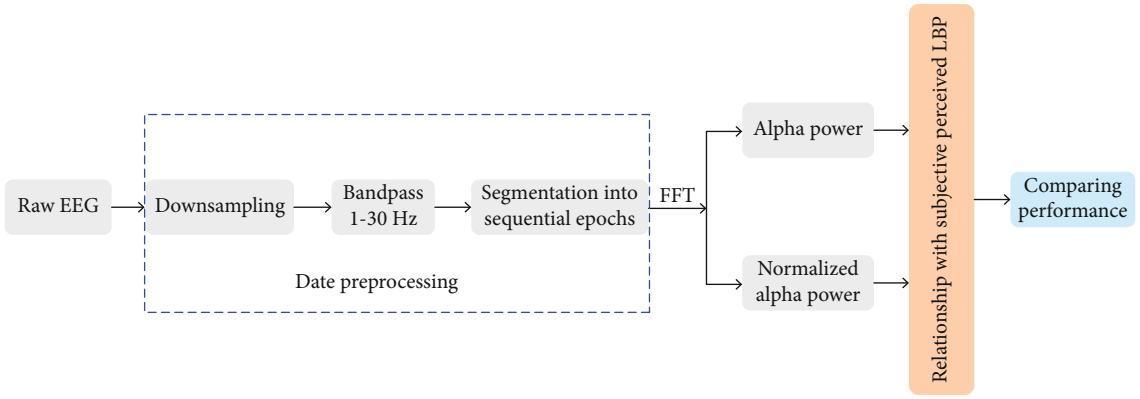

Figure 1: Flowchart describing the procedure used for EEG data analysis.

a band-pass filter in the range of 0.01–100 Hz and a sampling rate of 5000 Hz. An electrode placed on the nose was used as the reference channel, and the impedance at each electrode position was kept below 10 kΩ. Four surface electrodes were placed to record electrooculographic signals: one pair was placed lateral to the outer corner of the right and left orbit at a distance of 1 cm, and the other pair was placed over the upper and lower eyelids.

2.3. Experimental Procedure. Each subject was seated in a comfortable armchair in a quiet and temperature-controlled room. The subject was familiarized with the pain rating scale and the experimental procedures before the experiment, under clear guidance of an experienced physiotherapist. During the experiment, each subject began with the resting EC followed by EO baseline periods of 5 min each. For the EO condition, subjects were instructed to stay relaxed but alert. After the EEG recordings, subjects were asked to verbally rate their perceived pain intensity on a 0–10 VAS, in which 0 was defined as "no pain sensation" and 10 as "the worst imaginable pain".

2.4. EEG Data Analysis. The data analysis procedure in the present study is illustrated in Figure 1. Raw EEG data were first preprocessed by downsampling, band-pass filtering, and segmenting into sequential epochs. Then, spectral analysis was employed to obtain the spectral powers in the alpha band (both alpha power under the EO condition and normalized alpha power). Finally, the performance of different parameters for LBP assessment was compared by associating with subjective perceived LBP.

2.4.1. Data Preprocessing. Raw EEG data were processed in the MATLAB environment (MathWorks Inc., Massachusetts, United States). After downsampling the data to 1000 Hz, the data for each condition (EC and EO) were band-pass filtered in the range of 1–30 Hz. Then, each 5 min EEG recording was segmented into 300 sequential 1 s epochs, of which the first five were rejected to avoid evoked responses associated with the action of opening or closing the eyes. Each epoch was baselined across its duration and rejected if the activity at any scalp site exceeded ±100 μV at any time. EEG segments contaminated with strong muscle artifacts were manually eliminated by visual inspection. Epochs contaminated by eye blinks and movements were corrected using independent component analysis (ICA) in EEGLAB V13.0, an open-source toolbox running in the MATLAB environment. The main criteria to determine whether a component is artifact are the scalp map, the component time course, and the component activity power spectra [35]. In all datasets, independent components with a large electrooculogram (EOG) channel contribution and a frontal scalp distribution were removed. After preprocessing and selection, the remaining artifact-free, 1 s epochs from all 27 subjects were selected for further analyses.

2.4.2. Spectra Extraction. As the present study is aimed at investigating the relationship between alpha band activities and subjective perceived LBP, only data concerning the alpha frequency range (8-13 Hz) are presented. The segmented EEG epochs of each subject in each condition were transformed from the time domain to the frequency domain, using fast Fourier transformation (FFT), yielding power spectra (in μV^2). For each subject and electrode, the obtained power spectra over the frequency band were summed to summarize the spectral power values of the alpha band for each epoch; this procedure was repeated for all conditions. For each electrode, the obtained single-epoch power spectra were averaged across epochs to enhance the signal-to-noise ratio. Then, for each subject and condition, the power spectra were averaged in three regions: frontal (F3, Fz, F4, FC1, and FC2), central (C3, C1, Cz, C2, C4, CP1, CPz, and CP2), and posterior (P3, Pz, P4, O1, Oz, and O2). These electrodes were selected because of ample prior evidence indicating their substantial relevance in experimentally induced tonic pain [10, 24, 25, 27, 36].

Two calculations for the alpha power were performed to investigate various possible cortical parameters potentially associated with subjective perceived chronic LBP. The averaged alpha power value for each subject was obtained for the EC and EO conditions separately using the spectral analysis. The obtained values from the EO condition were utilized as one variable for correlation analysis to evaluate the relationship with the VAS scores. The second parameter investigated was the differences between the alpha band powers in the EC and EO conditions derived by alpha power

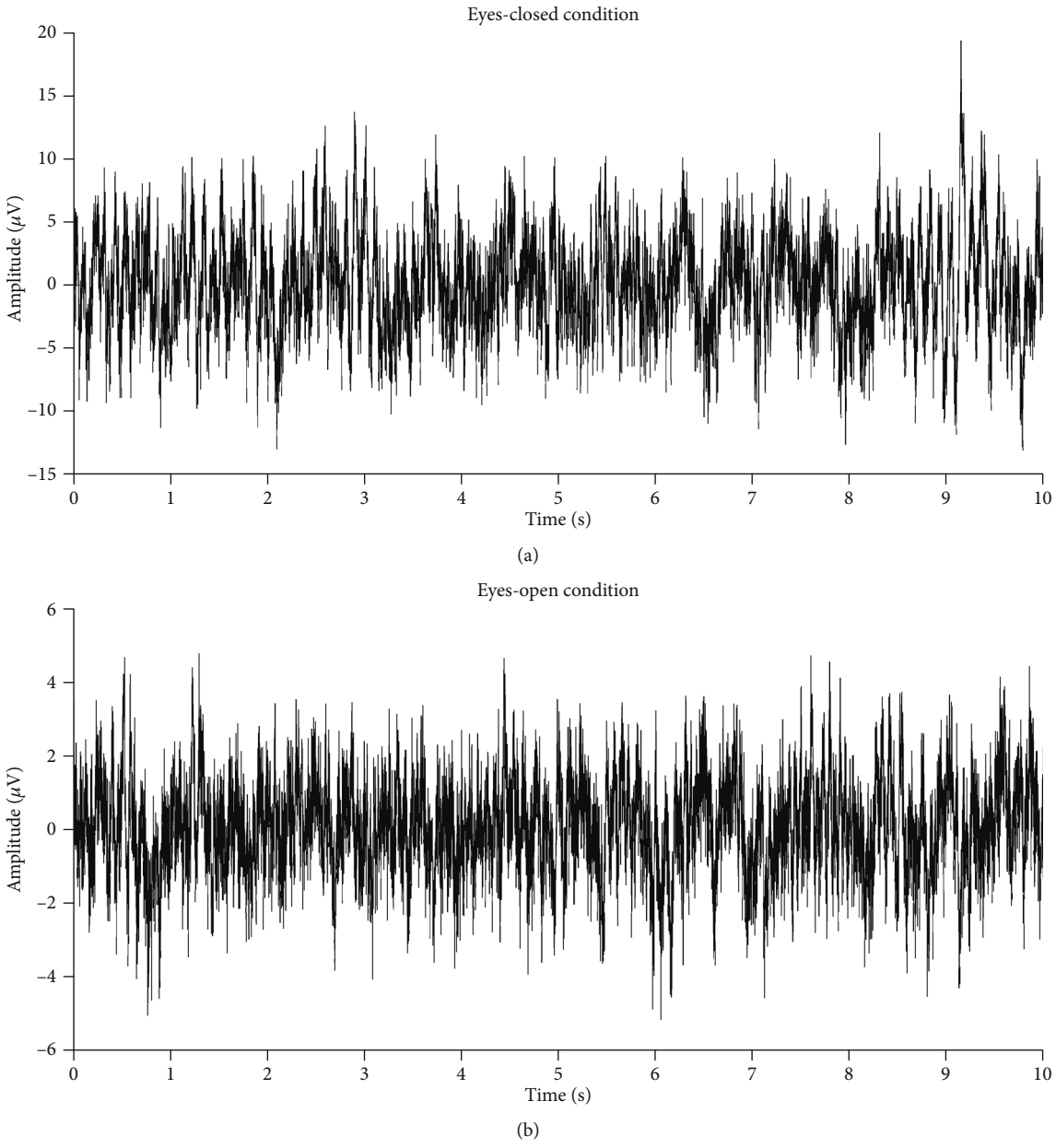

Figure 2: Representative 10 s long preprocessed EEG recordings derived from the Cz electrode in one randomly chosen subject under (a) eyes-closed (EC) and (b) eyes-open (EO) conditions.

normalization (here, the differences between the absolute alpha power values in the EC and EO conditions are the normalized alpha powers for statistical analysis).

2.4.3. Statistical Analysis. All statistical analyses were conducted in the MATLAB environment. To examine the global changes in alpha activity, the obtained power differences from each subject were group-averaged in the three main regions (frontal, central, and posterior). The mean and standard deviation (SD) were computed for each parameter.

The relationships between EEG responses and subjective pain intensities (VAS scores) were assessed by linear regression and Pearson correlation analyses, and the P values were corrected for multiple comparisons using the Bonferroni method. Specifically, two correlations were conducted to determine (1) whether the alpha power values obtained from resting EEG data are associated with subjective perceived chronic LBP and (2) which parameter (alpha power from the EO condition or normalized alpha power) is more strongly correlated with pain intensity. Thus, the first relationship to be assessed was between the alpha power values from the EO condition and VAS scores, and the second relationship to be assessed was between the absolute alpha power reduction from the EC to the EO condition and the VAS scores. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Spectral Power in Different Conditions. Figure 2 shows 10 s long preprocessed EEG signals derived from the Cz

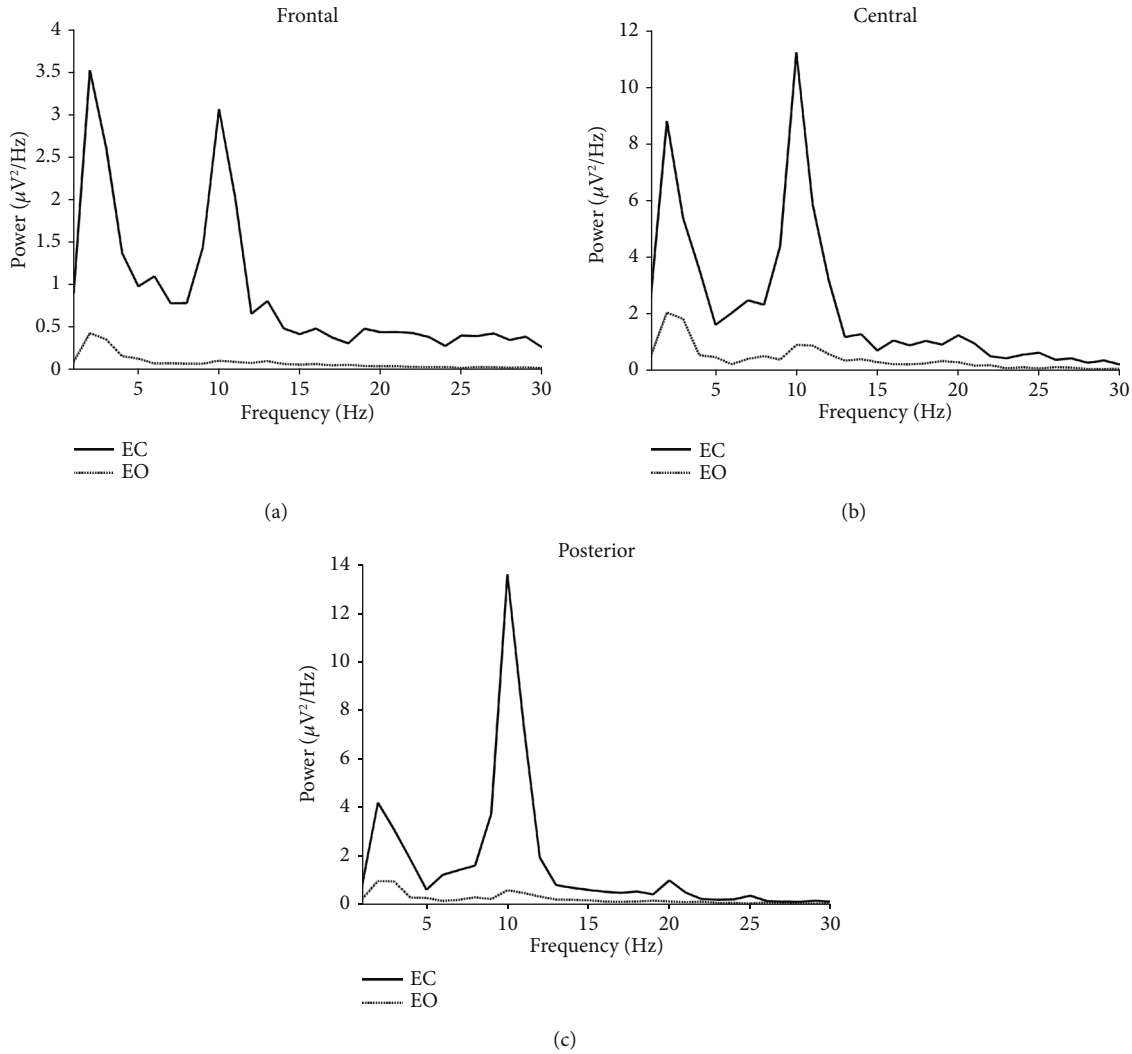

Figure 3: Group-averaged EEG power spectral densities under eyes-closed (EC) and eyes-open (EO) conditions in (a) frontal, (b) central, and (c) posterior regions.

electrode in one randomly chosen subject under the EC and EO conditions. Figure 3 shows the mean alpha power spectral densities under the EC and EO conditions averaged over the frontal (F3, Fz, F4, FC1, and FC2), central (C3, C1, Cz, C2, C4, CP1, CPz, and CP2), and posterior (P3, Pz, P4, O1, Oz, and O2) electrodes. The spectral power for the EO condition was significantly decreased compared with those of the EC condition within the alpha band in the three main regions.

3.2. Global Alpha Power Change between Conditions. To examine the global changes in alpha power, the absolute power differences in the alpha band at each electrode were calculated and then group-averaged over the frontal, central, and posterior regions. The topographic effects of the summarized spectral power reduction within the alpha band (8–13 Hz) are shown in Figure 4(a). The results show a global suppression in the alpha rhythms between the EC and EO conditions, especially in the occipital cortices. There was a local increase in the frontal area, but a decrease when computed over all electrodes. The means and SDs of the power differences for each subject in the three regions are shown in Figure 4(b). The group-averaged summarized alpha spectral power reduction was $0.36 \pm 0.20\,\mu V^2$ in the frontal region, $1.52 \pm 0.65\,\mu V^2$ in the central region, and $9.81 \pm 3.41\,\mu V^2$ in the posterior region.

3.3. Correlation between Alpha Power and Subjective Pain Rating. The correlations between the alpha spectral powers of each subject obtained in the EO condition and the subjective pain intensity are shown in Figure 5; no linear relationship was observed in any region ($P > 0.05$). The relationships between the power differences between EC and EO conditions and VAS scores are displayed in Figure 6. The reduction in the alpha power in the central region (C3, C1, Cz, C2, C4, CP1, CPz, and CP2) was negatively correlated with the subjective pain rating ($R = -0.513$, $P = 0.011$; Figure 6(a)), and the strongest correlation was

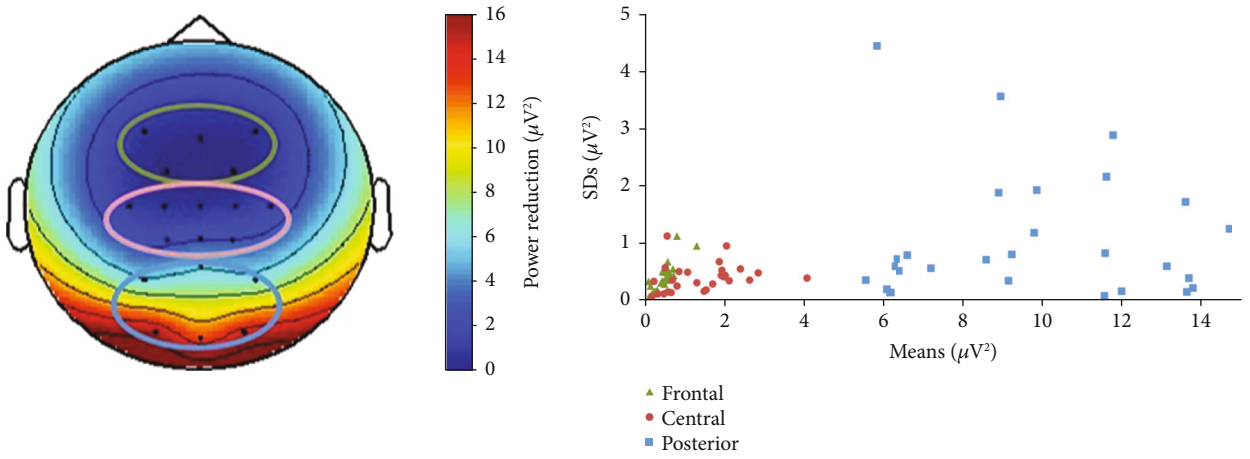

Figure 4: (a) Global power differences between eyes-closed (EC) and eyes-open (EO) conditions in the alpha frequency band and statistical results in the three main regions. (b) The means and SDs of the power differences for each subject in the three regions.

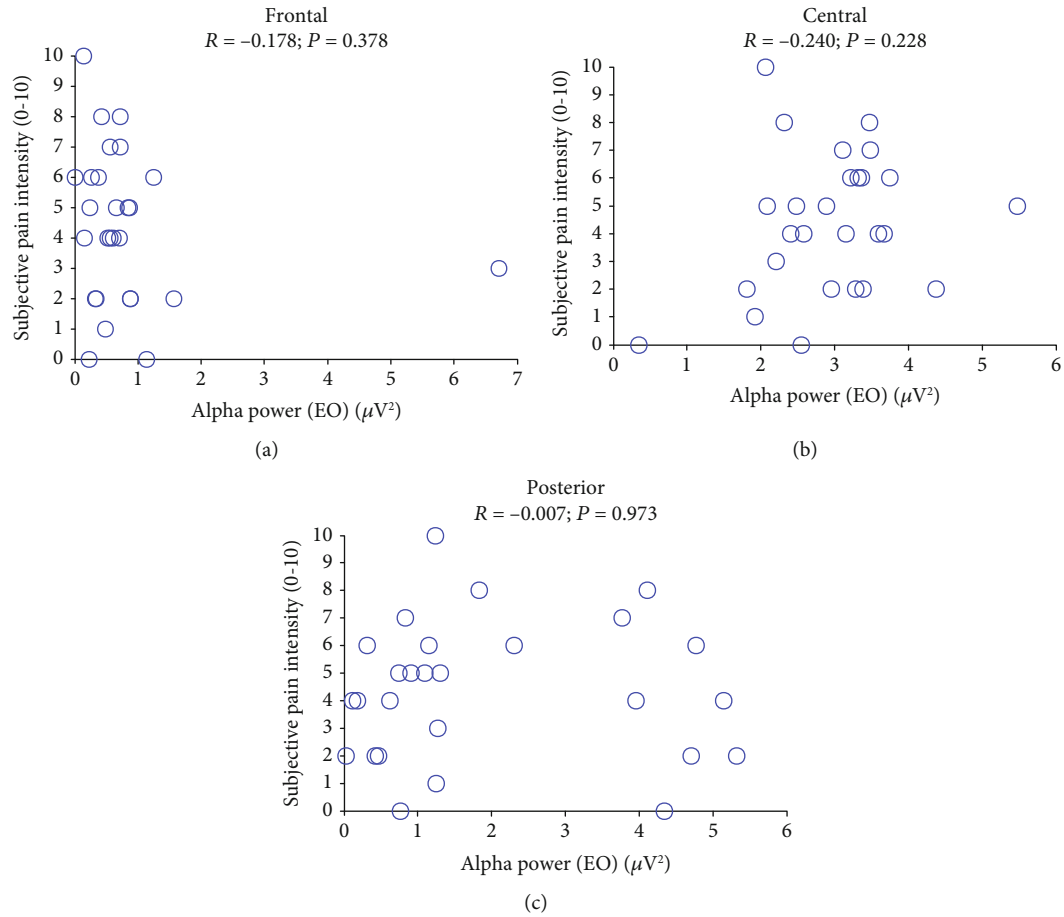

Figure 5: Relationships between alpha power under eyes-open (EO) condition and subjective intensity of pain perception in (a) frontal ($R = -0.178$, $P = 0.378$), (b) central ($R = -0.240$, $P = 0.228$), and (c) posterior ($R = -0.007$, $P = 0.973$) regions.

observed with the signal from the Cz electrode ($R = -0.590$, $P = 0.038$; Figure 6(b)). However, no such linear relationship was observed between the VAS scores and the alpha powers in the frontal ($R = -0.337$, $P = 0.086$; Figure 6(c)) or posterior electrodes ($R = 0.045$, $P = 0.825$; Figure 6(d)).

4. Discussion

In this study, we evaluate the relationship between the alpha power, an objective neurophysiological parameter, and subjective reports of experienced pain in people with chronic

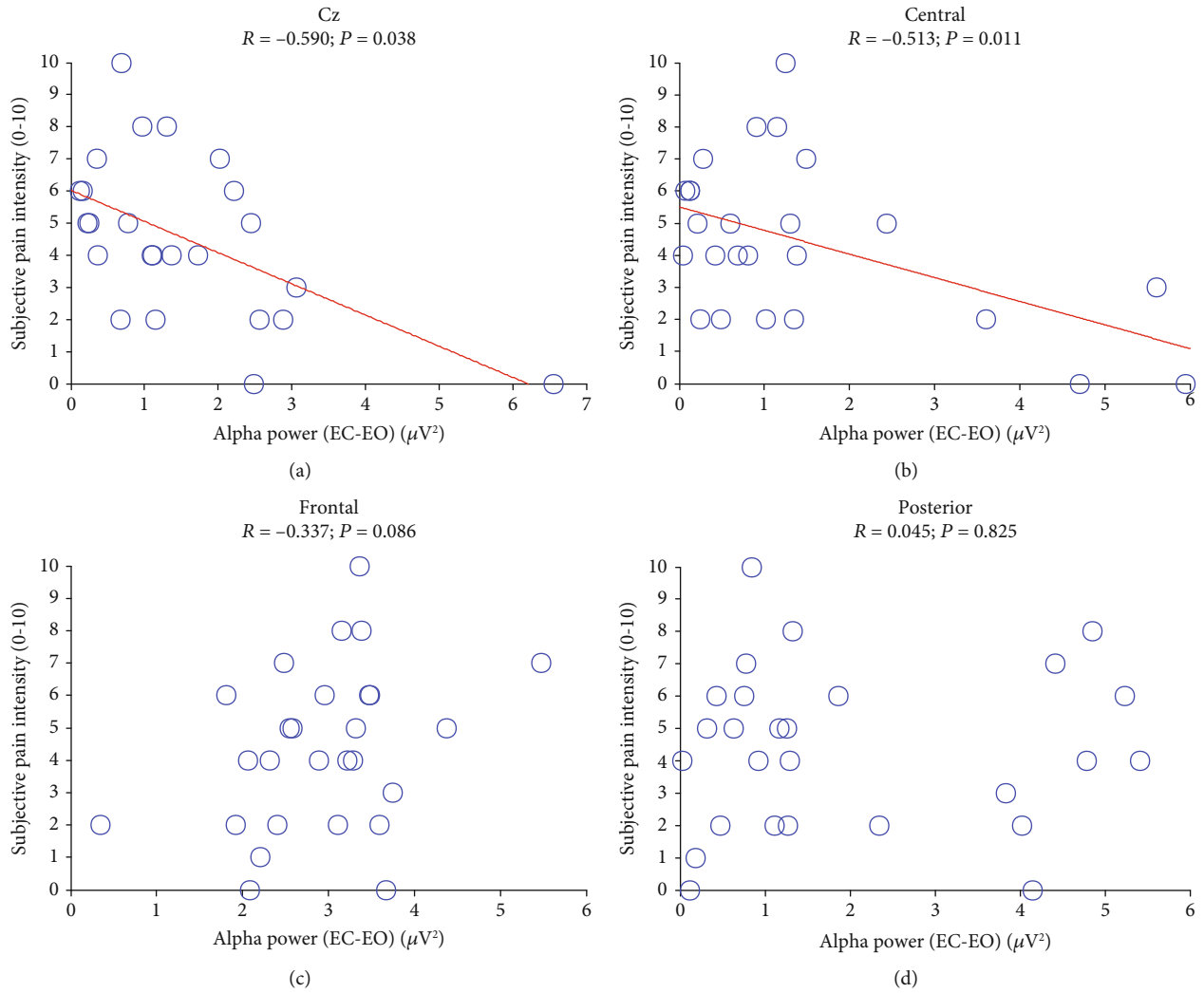

Figure 6: Relationships between alpha power reduction from eyes-closed (EC) condition to eyes-open (EO) condition and subjective intensity of pain perception in (a) central ($R = -0.513$, $P = 0.011$), (b) Cz electrode ($R = -0.590$, $P = 0.038$), (c) frontal ($R = -0.337$, $P = 0.086$), and (d) posterior ($R = 0.045$, $P = 0.825$) regions.

LBP. The observed decrease in alpha power in the central region under the conditions of closing and opening eyes in a resting state had a negative linear relationship with the subjective pain rating. Furthermore, the correlation between the normalized alpha power and VAS scores indicated that the alpha band spectral change between the EC and EO conditions from the resting EEG data might serve as a measurable and quantitative indicator for subjective perceived chronic pain.

For feature extraction within the alpha frequency band, we focus on the alpha spectral power in particular. Compared with other frequency domains, alpha band oscillations (8–13 Hz) are the most commonly explored, and the decrease in this metric has been repeatedly associated with the administration of noxious stimuli [15, 17, 22, 24, 27, 37]. It is noted that gamma oscillation features also have been reported to be related to pain perception and considered a potential pain assessment tool [14, 15, 18]. However, the signal-to-noise ratio of gamma oscillation is poor [27], since higher frequency data are normally and easily contaminated by a lot of nonneural artifacts (e.g., cranial and ocular muscle activity) with practical difficulty in clinical use, whereas alpha activity is related to the amount of experimentally induced pain in the somatosensory cortex, more reliably measured by scalp [18]. Based on the previous findings [32–34], to identify stable and reliable alpha band activities, the EC and EO conditions were evaluated experimentally. Using the simple modulation of closing and opening the eyes, the alpha power was directly obtained for each condition, and the power changes in the alpha band were obtained by computing the absolute power reductions between the conditions. The obtained power differences from each subject were group-averaged in the frontal, central, and posterior regions to evaluate global changes in the alpha activity. Statistical analyses of the means and SDs of the power changes showed a global suppression in the alpha rhythms from the EC to the EO condition. As observed in a study of healthy people [34], the alpha reduction in patients with chronic LBP was more

considerable in the posterior region, which suggests arousal in the EO condition compared with the EC condition. As described by Berger in the 1920s [38], the alpha rhythmic activity is the strongest electrophysiological signal measured from the surface of the awake human brain. High levels of alpha activity were previously interpreted as cortical idling because alpha activity increases in brain areas that are not engaged in a task.

To investigate whether alpha power is associated with subjective perceived chronic LBP and propose a more useful parameter, the relationships of the VAS scores with two different variables (alpha power in the EO condition and normalized alpha power) were investigated. The results of the correlation analysis showed that increased subjective perceived chronic LBP was solely associated with decreased oscillations of the alpha power between the EC and EO conditions (resting state) in the central region. Consistent with our findings, reductions in the alpha band were observed in previous works on tonic experimental pain using both heat- and cold-presser tests [10, 15, 27, 36, 39–42]. Compared with previous findings based on stimuli-evoked pain responses, the results of our study demonstrate that the suppression of alpha oscillations extracted from spontaneous EEG signals has a linear relationship with the subjective pain intensity in chronic LBP patients. This finding may indicate that alpha power differences between EC and EO conditions recorded at rest could reveal individual predispositions for pain responsiveness. Unlike previous studies [14, 15, 24, 27, 36, 39], in which EPs were induced by long-lasting stimuli in healthy subjects, we collected spontaneous resting EEG data from chronic LBP patients. Because this procedure does not require a task, the EEG data can be obtained much more readily from patients without training on performing specific tasks. Thus, the approach used here may serve as a simple and useful clinical methodology for measuring subjective pain intensity. In addition, an ongoing debate regarding nociception-associated changes in cortical oscillatory activity is whether the phenomena observed are elicited by pain or merely the saliency of external stimuli. Hu et al. [37] have shown that the alpha power fluctuations induced by phasic stimulation can reflect both sensory-related and task-related cortical processes, where sensory-related alpha event-related desynchronization (α-ERD) is predominantly located over contralateral central electrodes, and task-related α-ERD is located at posterior parietal and occipital electrodes. However, it remains unclear how tasks may affect the pain-induced changes in spontaneous oscillatory activity. Because the participants in our experiment were not asked to perform a task and did not receive any external stimuli, we can rule out the saliency effect.

Our results are also in accordance with EEG-based measurements performed in chronic pain patients, which found decreased cortical inhibition in pain-controlling regions [8]. However, some studies in patients with fibromyalgia, a disease in which mechanisms generating chronic pain are still elusive, reported increased or unchanged cortical inhibition [31, 43]. Other studies reported rather increased beta oscillations [16] and lower gamma oscillations [14] in neurogenic pain. Most of these studies were conducted using varied electrophysiological techniques and brain imaging methods, and the analysis frequency domain was segmented with different cutoffs. In addition, data acquired with eyes-closed condition were pooled together with those recorded in eyes-open condition, which is reported to dramatically modify both the topography and the magnitude of different frequency components [33]. In our study, selected data was recorded only in alpha frequency segmentation and normalized using validated methods [32–34]. The results imply that the proposed novel experimental setting used in the present study enables the effective extraction of more stable and reliable alpha oscillations than the traditional approach, which allows for meaningful correlations to be identified. Furthermore, this neurophysiological-psychophysical relationship broadens the existing knowledge regarding prolonged pain quantification based on alpha oscillations by delineating a distinct functional role of their power in representing subjective experiences of tonic pain in addition to their previously explored characteristics [37, 39, 44]. The identification of electrophysiological signatures encoding how the cortex processes the experience of chronic pain could indeed open a window to study the cortical process underlying the pain function in humans [45]. In clinical practice, this understanding also would make it possible to predict subjective pain intensity objectively and help explore the pathological mechanisms of chronic pain and achieve pain relief by modulating the oscillatory activities using neurofeedback techniques, with the investigation of cortical oscillatory activities on chronic pain patients [18].

The alpha power in the central region was the most responsive to changes in the subjective pain intensity. While it may be difficult to associate electrical activities recorded from the scalp with specific brain sources, their distribution is consistent with pain-related activities in the somatosensory association areas located in the parietal operculum and insula. Similar findings have been reported in the study of identifying oscillatory activities from intracerebral EEG by using tonic thermonociceptive stimulation [27, 36, 42]. Previous studies have characterized the cortical representation of pain in the primary sensorimotor areas (S1/M1) using intracortical evoked potentials from epileptic patients [46]. Our finding suggests that the central processing of chronic pain within an individual can be investigated through the neural function of their pain network at rest, enabling the prediction of subjective pain responsiveness without using external stimuli. This result also complements the report of alpha band amplitude study at the same location in patients with chronic pain [17]. Based on the close association between alpha oscillatory activity and cortical excitability [37, 44], the observed significant suppression in the central region (especially at the Cz electrode) may indicate elevated excitability in the sensorimotor cortex. Additionally, the general role of alpha rhythms in inhibiting different processes within the brain can be used as a framework to interpret the results [47, 48]. Suppressing alpha band power is thought to elicit a top-down feedback effect in the connectivity of different cortical regions, which allows for the information to flow from the sensory area to other relevant functional areas in response to pain [49, 50]. Moreover, the finding

revealed an increase in central processing in the case of chronic LBP, based on the close association between alpha oscillatory activity and cortical excitability [37, 44, 51].

However, even though our study has been carefully designed with a good clinical education and guidance for pain assessment to minimize individual differences, the individual variance such as personality or trait differences may cause subjective differences in pain perception [27]. The absence of a higher statistical power in the correlation results might be due to the effect of the outliers, considering the limited number of our trials. Additionally, in terms of our subjects' gender, we had a greater percentage of female participants. In the present study, we did not take gender balance as an influencing factor considering that the previous study did not observe any significant fixed effect from gender differences in EEG features of pain [22]. More balanced recruitment is expected to be conducted in the future to test gender effects.

5. Conclusions

In this study, we found that suppression of the alpha frequency band in the central electrodes under the conditions of closing and opening eyes in a resting state had a negative linear relationship with subjective perceived pain in chronic LBP patients. The degree of suppression may reflect the level of subjective pain intensity. These preliminary results, although needing confirmation, give the possibility for the objective and straightforward assessment of chronic pain by means of a short EEG recording at rest.

There were several limitations of the present work that could be addressed in future studies. First, as subjects were chronic low back pain patients, further investigation is needed to expand the current findings to other clinical pain populations. To increase the power of the approach and be able to detect reliable changes in alpha oscillations, more trials might be necessary. In addition, the mechanisms of underlying neural activity in chronic pain still need to be clarified. Extensive EEG source localization and connectivity analysis could be implemented to further explore these processes. Finally, although the present study focused on alpha band frequency due to their association with pain, additional bands should be considered to identify relationships between signals at other frequencies with subjective pain perception.

Data Availability

The datasets generated or analyzed during the current study are not publicly available due to the terms of consent to which the participants agreed but are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Y.H. performed the conceptualization. Y.H. and L.F. performed the methodology. L.F. and H.L. secured the software. H.C., X.X., and S.X. performed the validation. L.F. and H.L. contributed to the formal analysis. Y.H. contributed to the data curation. L.F. contributed to the writing—original draft preparation. L.F. and Y.H. performed the writing—review and editing. Y.H. and H.C. contributed to the funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Research Article

Ultrasound-Guided Transforaminal Injections of Platelet-Rich Plasma Compared with Steroid in Lumbar Disc Herniation: A Prospective, Randomized, Controlled Study

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Transforaminal steroid injection is extensively used as a treatment in cases of herniated disc, but it is associated with complications. In comparison, platelet-rich plasma (PRP) injection has been used in musculoskeletal disorders and could be another option. This study is aimed at comparing the efficacy and safety aspects between ultrasound-guided transforaminal injections of PRP and steroid in patients who suffer from radicular pain due to lumbar disc herniation. In a randomized controlled trial, ultrasound-guided transforaminal injections of either PRP ($n = 61$) or steroid ($n = 63$) were administered to a total of 124 patients who suffer from radicular pain due to lumbar disc herniation. Patients were assessed by the visual analogue scale (VAS), pressure pain thresholds (PPTs), Oswestry disability index (ODI), and the physical function (PF) and bodily pain (BP) domains of the 36-item short form health survey (SF-36) before operation and 1 week, 1 month, 3 months, 6 months, and 12 months after operation. The rate and latency of F-wave were obtained before operation and 12 months postoperation. There was no statistical difference in terms of age and sex between both groups. Statistically significant improvements from the patients' data before operation to data obtained 1-month postoperation were observed in VAS, PPTs, ODI, and PF and BP of SF-36 in both groups and kept for 1 year. F-wave rate and latency were improved significantly at 1-year postoperation in both groups. Intergroup differences during follow-ups over a period of 1 year were not found to be significant in all the above assessment between the PRP and steroid groups. No complications were reported. The results showed similar outcome for both transforaminal injections using PRP and steroid in the treatment of lumbar disc herniation, suggesting the possible application of PRP injection as a safer alternative. The trial was registered in the Chinese Clinical Trial Registry (ChiCTR-INR-17011825).

1. Introduction

Low back pain is one of the most difficult conditions to manage for doctors, patients, and policymakers. Not only does it limit physical activity, life quality is also greatly reduced alongside additional social and economic burden. The point prevalence of low back pain is 12%, with its one-year prevalence being 38% and the lifetime prevalence being approximately 40% [1]. Aging population leads to the rising number of individuals affected by low back pain. Lumbar disc herniation has been identified as the common etiology of low back pain [2]. The treatments for lumbar disc herniation vary from conservative to surgical management, which include analgesics, traction, physical therapy, manipulation, and psychotherapy. However, not all patients are able to be relieved from pain through these treatments.

For over 30 years, epidural steroid injection has been widely used as a treatment for lumbar disc herniation [3, 4], with its effectiveness proven by multiple research [5–7]. It works in anti-inflammation, pain relief, and functional improvement. There are three routes for steroid injection: interlaminar, transforaminal, and caudal routes. The transforaminal route fared better than the other two because it could reach the targeted sites, namely, spinal nerve, anterior epidural space, and the dorsal root ganglion, to counteract the inflammation secondary to compression [8]. However, there are still

concerns about the safety of epidural steroid injection. Based on literature, several complications related to epidural steroid injection have been pointed out, including neurotoxicity, pharmacologic effect of steroid (hypercorticism, adrenal suppression, and hyperglycemia), and neurologic injury [8, 9]. Besides, the contraindications of steroid use (allergy, diabetes, severe osteoporosis, pregnancy, severe hypertension, infection, etc.) limit the usage of epidural steroid injection.

Platelet-rich plasma (PRP), a biological product from the centrifugation of autologous blood with a high number of platelets in a small volume of plasma, has a positive effect on pain relief in some musculoskeletal diseases, especially osteoarthritis, tendinosis, and ligament tears [10]. PRP contains high concentration of growth factors (GFs) and cytokines that play important roles in anti-inflammatory, antiapoptotic, and proliferative effects on the neurons and fibroblasts [11]. Although the role of PRP in pain relief looks promising, the effect of transforaminal PRP injection in lumbar disc herniation with radicular pain remains unclear.

Henceforth, this study is aimed at investigating the efficacy and safety of ultrasound-guided platelet-rich plasma injections compared with steroid injections in treating lumbar disc herniation with radicular pain.

2. Materials and Methods

2.1. Study Design and Participant Recruitment. A prospective, randomized, and controlled study was carried out to compare the treatment of lumbar disc herniation with radicular pain with ultrasound-guided transforaminal steroid or PRP injections. This study was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital, and the trial was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR-INR-17011825). This investigation was conducted in accordance with the Declaration of Helsinki. 184 patients were assessed for eligibility based on the inclusion and exclusion criteria listed as follows.

Inclusion criteria are as follows: (1) aged 20-60 years; (2) low back pain with unilateral lower limb radicular pain, duration of more than 3 months; (3) posterolateral lumbar disc herniation of L4/L5 or L5/S1segment diagnosed by MRI or CT and consistent with the clinical symptoms and signs; (4) degree of pain (VAS) more than 5 and obvious symptoms and clinical signs of nerve root irritation; (5) had received conservative treatment, including physical therapy, manipulation, and nonmorphine treatment; (6) no symptoms of severe nerve damage including motor paralysis, muscle atrophy, and cauda equina syndrome; (7) had no history of spinal surgery.

Exclusion criteria are as follows: (1) infection; (2) had received prior injection treatment in the past 3 months, such as nerve root injection and caudal injection; (3) spinal tumors or tuberculosis; (4) multisegmental lumbar disc herniation, spinal deformity, or spinal stenosis; (5) not suitable for local injection; (6) allergic to the drug used in this study; (7) a history of drug abuse or oral anticoagulation; (8) pregnancy; (9) severe diabetes; (10) clinical diagnosis of heart disease, liver and kidney dysfunction, and hematological diseases; (11) abnormal psychological and cognitive disorders.

52 patients were not enrolled in this study (31 patients either did not meet the inclusion criteria or met the exclusion criteria, while the other 21 patients declined to participate). The randomization sequence was produced by a statistician, who did not contact with patients, using a random number generator. The other 132 patients who were enrolled were simply randomized at a ratio of 1 : 1 to 2 groups: the steroid group (control group, $n = 68$) and the PRP group (experimental group, $n = 64$). The randomization was performed by the nurse, who did not participate in the process of patient assessment, by opening the numbered sealed opaque envelop. A written informed consent was obtained from each patient before enrollment. During follow-ups over 1 year, 4 patients did not receive allocated intervention and 4 others did not follow through. Thus, only 124 patients were included for data analysis (steroid group $n = 63$, PRP group $n = 61$) (Figure 1).

2.2. Study Procedure. The patients who were enrolled to this study underwent physical examination, neurological examination, and laboratory tests. The operation was performed at the operating room in the rehabilitation medicine department of Sun Yat-sen Memorial Hospital.

The procedure of PRP preparation was as follows: 18 ml blood sample was drawn from the anterior elbow vein and mixed with 2 ml of 3.8% (w/v) sodium citrate. The blood sample was then centrifuged at 1600 rpm for 10 minutes at room temperature (RT, 23°C) under aseptic condition to divide the sample into 3 layers. The lower layers composed of red blood cells were subsequently removed. The remaining sample was transferred into a new centrifuge tube and was centrifuged again at 3200 rpm for 10 minutes at RT. 4 ml was collected from the lower part which contains PRP. 1 ml of this PRP was then sent for quantitative analysis of platelet count.

The procedure of operation was as follows. An experienced physician performed the ultrasound-guided transforaminal injection using an ultrasonic device (Konica Minolta Medical & Graphic (Shanghai) Company limited, SONIMAGE HS1 PLUS, Tokyo, Japan) in out-of-plane approach. The patients were prepared in prone position with a pillow under their abdomen. The area of injection was disinfected. The sacral spinous process and the fifth lumbar spinous process transducer were identified when transducer placed longitudinally. The transducer in midline was moved laterally to recognize the lamina, transverse process, and facet joint. Then, the edge of the zygapophyseal joints was obtained when the transducer was moved back. The needle (22 G) was subsequently inserted into the skin using the out-of-plane approach upon determination of injection level to ensure that the needle tip was positioned in the middle of adjacent facet joints. After an inhalation test yielding negative results for cerebrospinal fluid and blood aspiration, the injection was administered (steroid group: 2 ml betamethasone+0.5 ml 0.9% sterile saline+0.5 ml 2% lidocaine; PRP group: 3 ml autologous PRP) (Figure 2). The detail of the ultrasound-guided out-of-plane injection can be found in this reference [12].

In the follow-up, some short-acting analgesics were given to patients when they felt obvious pain. The patients would

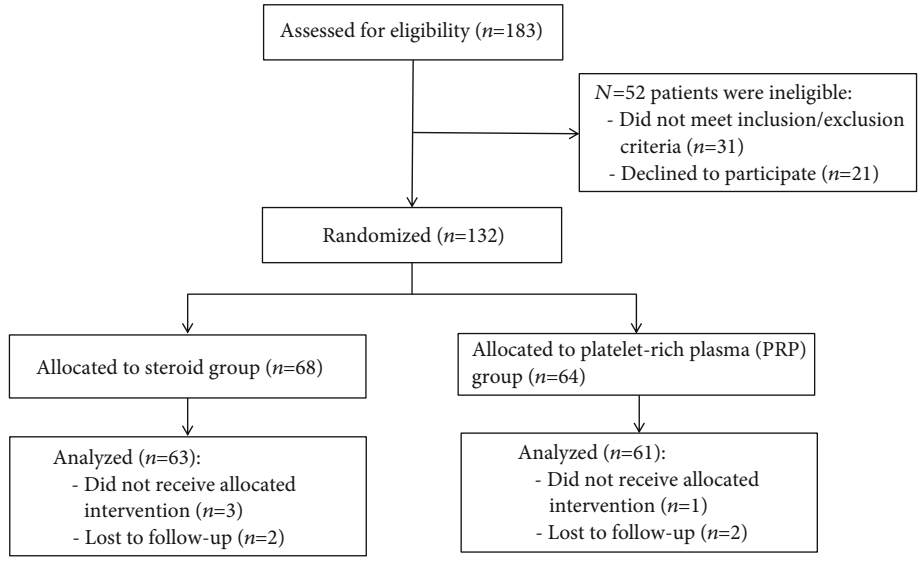

Figure 1: Flow diagram of enrolment, randomization, and analysis.

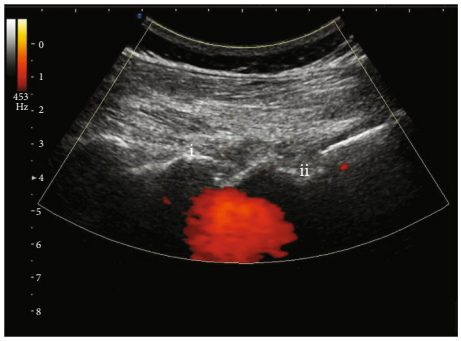

Figure 2: Ultrasound-guided transforaminal injection in out-of-plane approach. Ultrasound-guided transforaminal injection was performed, and the needle tip was positioned in the middle of (i) L5/S1 facet joint and (ii) sacral foramina. The red signal represented the spreading of drug in targeted epidural space.

be excluded from this study in follow-up when patients had any one of the following situations: (1) had overloaded pain which affects their life quality; (2) had symptom of motor paralysis, muscle atrophy, or cauda equina syndrome in the follow-up period; (3) transforaminal epidural injection failed to improve pain or function of patients during 3 months postoperation. Treatments including surgery or conservative treatments were given to those excluded patients.

2.3. Assessment and Outcome. The statistics of demographic characteristics, including gender and age, as well as baseline information of patients were collected upon admission of the patients. Baseline information was obtained before operation, which includes visual analogue scale (VAS), pressure pain thresholds (PPTs), the rate and latency of F-wave, Oswestry disability index (ODI), and physical function (PF) and bodily pain (BP) from the 36-item short form health survey (SF-36). Each patient was required to complete the same assessments as the baseline information (except F-wave rate and latency) at 1 week, 1 month, 3 months, 6 months, and 1 year postoperation. F-wave rate and latency were obtained only 1 year postoperation. All assessments were independently performed by two experienced blinded doctors.

The visual analogue scale (VAS) was a method to evaluate the degree of pain. A 10 cm line was used as an indicator whereby one end represents no pain, while the other end represents the most severe pain imagined. The patient was asked to indicate the point on the line which could represent the patient's pain level [13].

The pressure pain thresholds (PPTs) were measured by an algometry device (Pain Diagnostics and Thermography Corporation, Model PTH AF2, Great Neck, NY 11023) according to the procedure recommended by Fischer [14]. A plastic tip was placed at the paraspinal tenderness point in the segment with lumbar disc herniation. Detailed description can be found in our previous article [15].

The rate and latency of F-wave in the affected side were measured by electromyogram. The recording electrode was placed at the muscle belly of abductor halluces of the affected side; the reference electrode was placed at the tendon of abductor halluces; the stimulation electrode was placed at the tibial nerve behind the medial malleolus; the ground wire was placed at the ankle joint between the stimulation electrode and the recording electrode. At stimulation points of the affected side, 20 consecutive stimuli at the frequency of 1 Hz and the width of 0.2 ms were induced, obtaining records of the rate and latency of F-waves at the affected sides.

There were 10 items in the Oswestry disability index (ODI), including pain, individual function, and personal comprehensive function. The minimum score for each item is 0 (good state), whereas the highest score is 5 (poor state). The Oswestry disability index referred to the percentage of the sum of score from all 10 items out of 50.

The 36-item short form health survey (SF-36) consisted of 36 items, which includes one scale on health transition and 8 domains. The score for each domain ranges from 0 (poor health) to 100 (good health). The reliability, validity, and application of the Chinese version SF-36 have been

Table 1: Demographic characteristics and baseline information of patients.

	Steroid group ($n = 63$)	PRP group ($n = 61$)	P value
Age (y, median (1^{st}-3^{rd}))	56.0 (50.0-59.0)	56.0 (44.5-60.0)	0.910
Female (N (%))	26 (41.3)	33 (54.1)	0.153
VAS (median (1^{st}-3^{rd}))	6.0 (5.0-7.0)	6.0 (6.0-7.25)	0.106
PPTs (kPa, median (1^{st}-3^{rd}))	598.74 (535.24-607.81)	580.60 (557.92-601.01)	0.703
F-wave rate (%, median (1^{st}-3^{rd}))	82.0 (80-95)	82.0 (80.0-85.0)	0.161
F-wave latency (ms, median (1^{st}-3^{rd}))	48.9 (47.8-50.8)	48.7 (46.9-49.7)	0.217
ODI (%, median (1^{st}-3^{rd}))	27.0 (21.0-43.0)	35.0 (26.35-44.0)	0.193
PF of SF-36 (median (1^{st}-3^{rd}))	65.0 (55.0-80.0)	60.0 (45.0-70.0)	0.091
BP of SF-36 (median (1^{st}-3^{rd}))	41.0 (41.0-52.0)	41.0 (31.0-51.0)	0.428

PRP: platelet-rich plasma; 1st-3rd: 1st-3rd quartiles; PPTs: pressure pain thresholds; VAS: visual analogue scale; ODI: Oswestry disability index; SF-36: the 36-item short form health survey; PF: physical function; BP: bodily pain.

proven [16]. In this study, two domains were recorded, namely, the physical function (PF) and bodily pain (BP) domains.

2.4. Statistical Analysis. With a sample size of 60 patients each group, we calculated that the study would have the power of 80% to detect a 0.9 difference at the significant improvement in VAS between groups from baseline to 6 months with the 2 standard deviation of the change in VAS. The mean and standard deviation were based on data from previous literature and our previous study [17, 18]. Besides, there was an increase of 15% in sample size in each group in case of the loss to follow-up. Two-sided α level was 0.05.

Data were analyzed using the SPSS 23.0 software. The continuous variables were expressed by mean ± standard deviation or medians (1st-3rd quartiles) depending on data distribution, while the discrete variable (such as sex) was described as n (%). The Shapiro-Wilk test was used to test the data distribution of the continuous variables. Continuous data before operation with nonnormal distribution was analyzed using the Mann–Whitney U test, whereas the discrete variable was analyzed by the χ^2/Fisher exact test. To compare the difference between the steroid group and the PRP group over time, the generalized estimating equation was used to estimate the time × treatment interaction. A negative interaction represents the ability of the generalized estimating equation in indicating the difference between the steroid and PRP groups over time. If the interaction existed, the Mann–Whitney U test can be used to compare the difference between both groups at the same time point. The Friedman test was used to evaluate the difference between different time points within one group, while the post hoc test was used to compare the data between preoperation and postoperation in one group. The Wilcoxon signed-rank test was used for paired sample in one group. The difference is considered statistically significant when bilateral $\alpha = 0.05$, $P < 0.05$.

3. Results

3.1. Patient Characteristics. There were 124 patients who completed follow-ups over the period of 1 year with 63 patients in the steroid group and 61 patients in the PRP group. There was no statistically significant difference in age and gender between both groups ($P > 0.05$) (Table 1). Besides, no statistically significant differences were found in VAS, PPTs, F-wave rates and latency, ODI, and physical function (PF) and bodily pain (BP) domains of SF-36 between the two groups before operation ($P > 0.05$) (Table 1).

3.2. PRP Longitudinal Data. The PRP group consisted of 61 participants who were enrolled and completed the follow-ups over 1 year. There was no significant difference in terms of VAS, PPTs, ODI, and the PF and BP domains of SF-36 in 1 week postoperation compared to corresponding basal values (median (1st-3rd quartiles); 6.0 (6.0-7.3) vs. 5.0 (5.0-6.0), $P = 0.887$; 580.60 kPa (557.92-601.01) vs. 625.96 kPa (571.53-716.68), $P = 0.087$; 35.0% (26.4-44.0) vs. 27.0% (20.0-40.0), $P = 0.125$; 60.0 (45.0-70.0) vs. 75.0 (60.0-90.0), $P = 0.284$; 41.0 (31.0-51.0) vs. 43.0 (41.0-52.0), $P = 0.794$, respectively). Statistically significant improvements were observed in terms of VAS, PPTs, ODI, and the PF and BP domains of SF-36 in 1-month postoperation compared to corresponding basal values (Tables 2 and 3). The median (1st-3rd quartiles) VAS was 6.0 (6.0-7.3), which decreased significantly to 3.0 (3.0-4.0) 1 month after operation ($P < 0.001$). Over the same period of time, PPTs had also significantly improved from 580.60 kPa (557.92-601.01) to 707.60 kPa (612.35-780.18) ($P < 0.001$), and ODI reduced from 35.0% (26.4-44.0) to 22.0% (14.25-42.5) ($P < 0.001$). The PF and BP domains of SF-36 had also significantly improved from baseline (60.0 (45.0-70.0) and 41.0 (31.0-51.0), respectively) to 1 month postoperation (88.0 (76.5-95.0), $P < 0.001$; 52.0 (41.0-62.0), $P < 0.001$, respectively). In the PRP group, statistically significant differences were also observed between baseline VAS, PPTs, ODI, and PF and BP domains of SF-36 and the same set of data from 3 months, 6 months, and 1 year postoperation (Tables 2 and 3). F-wave rate was higher after the administration of transforaminal PRP injection, increasing from 82.0% (80.0%-85.0%) to 95.0% (92.0%-100.0%) 1 year after operation ($P < 0.001$). Significant decrease in F-wave latency was observed in the PRP group, where it decreases from 48.7 ms (46.9-49.7) to 45.2 ms (44.5-46.2) 1 year postoperation ($P < 0.001$) (Table 3).

Table 2: Longitudinal outcomes of pain degree and spinal function for the PRP group over time.

Outcome	Time	PRP group ($n = 61$)	P value[#]
VAS (median (1^{st}-3^{rd}))	Baseline	6.0 (6.0-7.3)	Ref
	1 week	5.0 (5.0-6.0)	0.887
	1 month	3.0 (3.0-4.0)	<0.001
	3 months	3.0 (3.0-3.0)	<0.001
	6 months	3.0 (2.0-3.0)	<0.001
	1 year	2.0 (1.0-3.0)	<0.001
	P value over time[↑]	<0.001	
PPTs (kPa, median (1^{st}-3^{rd}))	Baseline	580.60 (557.92-601.01)	Ref
	1 week	625.96 (571.53-716.68)	0.087
	1 month	707.60 (612.35-780.18)	<0.001
	3 months	725.75 (698.53-843.68)	<0.001
	6 months	725.75 (694.00-823.27)	<0.001
	1 year	730.28 (694.00-789.25)	<0.001
	P value over time[↑]	<0.001	
ODI (%, median (1^{st}-3^{rd}))	Baseline	35.0 (26.4-44.0)	Ref
	1 week	27.0 (20.0-40.0)	0.125
	1 month	22.0 (14.3-42.5)	<0.001
	3 months	20.0 (16.5-29.0)	<0.001
	6 months	20.0 (14.0-29.0)	<0.001
	1 year	19.0 (15.5-30.0)	<0.001
	P value over time[↑]	0.001	

PRP: platelet-rich plasma; 1st-3rd: 1st-3rd quartiles; PPTs: pressure pain thresholds; VAS: visual analogue scale; ODI: Oswestry disability index. [#]P value compares difference from baseline using post hoc test or Wilcoxon signed-rank test. [↑]P value indicates significance of overall change over time using the Friedman test.

3.3. Steroid Longitudinal Data. The steroid group consisted of 63 patients who completed all the follow-up sessions over 1 year. There was no significant difference in terms of VAS, PPTs, ODI, and the PF and BP domains of SF-36 in 1 week postoperation compared to corresponding basal values (median (1st-3rd quartiles); 6.0 (5.0-7.0) vs. 6.0 (5.0-6.0), $P = 1.000$; 598.74 kPa (535.24-607.81) vs. 598.74 kPa (526.17-694.00), $P = 0.683$; 27.0% (21.0-43.0) vs. 23.0% (20.0-40.0), $P = 0.645$; 65.0 (55.0-80.0) vs. 70.0 (60.0-90.0), $P = 0.152$; 41.0 (41.0-52.0) vs. 47.0 (41.0-61.0), $P = 1.000$, respectively). Significant improvement was observed from baseline VAS, PPTs, ODI, and PF and BP domains of SF-36 to the same set of data from 1 month postoperation (Tables 4 and 5). During this period of time, VAS decreased from 6.0 (5.0-7.0) to 3.0 (3.0-5.0) ($P < 0.001$); PPTs increased from 598.74 kPa (535.24-607.81) to 598.74 kPa (526.17-694.00) ($P < 0.001$); ODI significantly decreased from 27.0% (21.0-43.0) to 18.0% (12.0-29.0) ($P < 0.001$), whereas the PF and BP domains of SF-36 all improved statistically from baseline (65.0 (55.0-80.0) and 41.0 (41.0-52.0), respectively) to 1 month postoperation (88.0 (75.0-95.0), $P < 0.001$; 52.0 (41.0-72.0), $P = 0.004$, respectively). Statistical differences were found between baseline VAS, PPTs, ODI, and PF and BP domains of SF-36 in the steroid group and data obtained from 3 months, 6 months, and 1 year postoperation (Tables 4 and 5). The F-wave rate had significantly increased from 82.0% (80.0-95.0) to 95.0% (90.0-100.0) 1 year postoperation ($P < 0.001$), while the F-wave latency had significantly decreased from 48.9 ms (47.8-50.8) to 45.2 ms (43.6-46.3) 1 year post operation ($P < 0.001$) (Table 5).

3.4. Intergroup Differences. During the 1-year follow-up period in this study, both the PRP and steroid groups demonstrated obvious improvements in terms of VAS score (Figure 3(a)), PPTs (Figure 3(b)), F-wave rate (Figure 4(a)) and latency (Figure 4(b)), ODI (Figure 3(c)), and the PF (Figure 3(d)) and BP (Figure 3(e)) domains of SF-36. Anyhow, intergroup differences during this 1-year follow-up period were not found to be significant in all tests involved (Figures 3 and 4).

3.5. Safety. No complications or adverse effects were reported after the ultrasound-guided transforaminal injection of PRP or steroid during the 1-year follow-up period.

4. Discussion

This study shows that ultrasound-guided transforaminal injection of both PRP and steroid leads to the significant improvement in the aspects of pain relief, nerve repair, spinal function, and life quality. Furthermore, the outcome after one whole year of follow-up has proven that these improvements stay effective for long term. Besides, no complications or side effects were found during any of the follow-ups.

Results from this study are in accordance with a series of clinical studies which have described the effectiveness of

Table 3: Longitudinal outcomes of life quality and nerve function for the PRP group over time.

Outcome	Time	PRP group ($n = 61$)	P value[#]
PF of SF-36 (median (1^{st}-3^{rd}))	Baseline	60.0 (45.0-70.0)	Ref
	1 week	75.0 (60.0-90.0)	0.284
	1 month	88.0 (76.5-95.0)	<0.001
	3 months	90.0 (82.5-95.0)	<0.001
	6 months	90.0 (87.5-93.0)	<0.001
	1 year	90.0 (90.0-95.0)	<0.001
	P value over time[↑]	<0.001	
BP of SF-36 (median (1^{st}-3^{rd}))	Baseline	41.0 (31.0-51.0)	Ref
	1 week	43.0 (41.0-52.0)	0.794
	1 month	52.0 (41.0-62.0)	0.005
	3 months	82.0 (61.0-94.0)	<0.001
	6 months	74.0 (62.0-85.5)	<0.001
	1 year	74.0 (64.0-87.0)	<0.001
	P value over time[↑]	<0.001	
F-wave rate (%, median (1^{st}-3^{rd}))	Baseline	82.0 (80.0-85.0)	Ref
	1 year	95.0 (92.0-100.0)	<0.001
F-wave latency (ms, median (1^{st}-3^{rd}))	Baseline	48.7 (46.9-49.7)	Ref
	1 year	45.2 (44.5-46.2)	<0.001

PRP: platelet-rich plasma; 1st-3rd: 1st-3rd quartiles; SF-36: the 36-item short form health survey; PF: physical function; BP: bodily pain. [#]P value compares difference from baseline using post hoc test or Wilcoxon signed-rank test. [↑]P value indicates significance of overall change over time using the Friedman test.

Table 4: Longitudinal outcomes of pain degree and spinal function for the steroid group over time.

Outcome	Time	Steroid group ($n = 63$)	P value[#]
VAS (median (1^{st}-3^{rd}))	Baseline	6.0 (5.0-7.0)	Ref
	1 week	6.0 (5.0-6.0)	1.000
	1 month	3.0 (3.0-5.0)	<0.001
	3 months	3.0 (2.0-4.0)	<0.001
	6 months	2.0 (2.0-3.0)	<0.001
	1 year	2.0 (1.0-3.0)	<0.001
	P value over time[↑]	<0.001	
PPTs (kPa, median (1^{st}-3^{rd}))	Baseline	598.74 (535.24-607.81)	Ref
	1 week	598.74 (526.17-694.00)	0.683
	1 month	739.36 (607.81-807.39)	<0.001
	3 months	739.36 (698.53-943.47)	<0.001
	6 months	725.75 (694.00-780.18)	<0.001
	1 year	716.68 (694.00-762.04)	<0.001
	P value over time[↑]	<0.001	
ODI (%, median (1^{st}-3^{rd}))	Baseline	27.0 (21.0-43.0)	Ref
	1 week	23.0 (20.0-40.0)	0.645
	1 month	18.0 (12.0-29.0)	<0.001
	3 months	20.0 (12.0-29.0)	<0.001
	6 months	20.0 (16.3-29.0)	<0.001
	1 year	20.0 (17.3-40.0)	<0.001
	P value over time[↑]	0.001	

1st-3rd: 1st-3rd quartiles; PPTs: pressure pain thresholds; VAS: visual analogue scale; ODI: Oswestry disability index. [#]P value compares difference from baseline using post hoc test or Wilcoxon signed-rank test. [↑]P value indicates significance of overall change over time using the Friedman test.

Table 5: Longitudinal outcomes of life quality and nerve function for the steroid group over time.

Outcome	Time	Steroid group ($n = 63$)	P value[#]
PF of SF-36 (median (1^{st}-3^{rd}))	Baseline	65.0 (55.0-80.0)	Ref
	1 week	70.0 (60.0-90.0)	0.152
	1 month	88.0 (75.0-95.0)	<0.001
	3 months	90.0 (70.0-95.0)	<0.001
	6 months	90.0 (88.0-95.0)	<0.001
	1 year	90.0 (80.0-95.0)	<0.001
	P value over time[↑]	<0.001	
BP of SF-36 (median (1^{st}-3^{rd}))	Baseline	41.0 (41.0-52.0)	Ref
	1 week	47.0 (41.0-61.0)	1.000
	1 month	52.0 (41.0-72.0)	0.004
	3 months	74.0 (51.0-94.0)	<0.001
	6 months	72.0 (62.0-94.0)	<0.001
	1 year	74.0 (62.0-94.0)	<0.001
	P value over time[↑]	<0.001	
F-wave rate (%, median (1^{st}-3^{rd}))	Baseline	82.0 (80.0-95.0)	Ref
	1 year	95.0 (90.0-100.0)	<0.001
F-wave latency (ms, median (1^{st}-3^{rd}))	Baseline	48.9 (47.8-50.8)	Ref
	1 year	45.2 (43.6-46.3)	<0.001

1st-3rd: 1st-3rd quartiles; SF-36: the 36-item short form health survey; PF: physical function; BP: bodily pain. [#]P value compares difference from baseline using post hoc test or Wilcoxon signed-rank test. [↑]P value indicates significance of overall change over time using the Friedman test.

epidural PRP injections for treating lumbar disc herniation with radicular pain [19–21]. In a nonrandomized comparative trial performed by Bise et al. on 60 patients with lumbar radicular pain in 2020, the CT-guided epidural PRP injection therapy was shown to cause significant pain reduction and functional improvement, which were measured using the numerical rating scale (NRS) and the Oswestry disability index (ODI). The effects of PRP injection were sustained for 6 weeks with no complications reported [19]. Centeno et al. investigated the efficacy of C-arm fluoroscopy-guided epidural platelet lysate injections in patients with lumbar radicular pain and found significant improvement in pain and function from baseline data to data obtained throughout the 2 years of follow-up period, suggesting the potential of PRP as a promising alternative to epidural steroids [20]. In this study, the PF and BP domains from SF-36 showed significant improvement at 1 month after operation and persisted for at least one year in the PRP group. However, there is no statistically significant difference between the PRP group and the steroid group in terms of the PF and BP domains of SF-36. In a randomized, controlled, and double-blinded study performed on 50 patients with complex chronic degenerative spinal pain, Ruiz-Lopez et al. found similar improvement with SF-36 scores measured at 6 months in the PRP group of fluoroscopically guided caudal epidural injection, whereas the steroid group only showed improvement in the BP of SF-36 [22]. Variation in routes of epidural injection and the type of degenerative spinal pain may attribute to these differences in the results.

F-wave measurement helps to assess conduction of impulse along the peripheral motor nerve, including most of its proximal segment. This investigation revealed the significant decrease in F-wave latencies and increase of F-wave rate in both the PRP and steroid groups, indicating the nerve repair function of PRP and steroid. Steroid has positive effects on the F-wave in peripheral nerve disorder, such as chronic inflammatory demyelinating polyradiculopathy and Guillain-Barré syndrome. One study reported that local steroid injection could help to decrease the F-wave latency in carpal tunnel syndrome [23]. Although no studies were found to support the claim about the impact of epidural PRP injection on F-wave latency and the rate in lumbar radicular pain, several studies have reported the positive role of PRP in nerve healing and reduction of neuropathic pain [24, 25]. Anjayani et al. investigated the efficacy of PRP injection in leprosy peripheral neuropathy, which shows the effect of PRP on nerve regeneration and improvement of peripheral neuropathy sensibility [24].

In recent years, PRP has been widely used in treating musculoskeletal diseases due to its anti-inflammatory properties and ability in promoting the processes of endogenous healing by delivering a high concentration of growth factors and cytokines [23, 26]. These growth factors, such as vascular endothelial growth factor (VEGF), transforming growth factor β-1 (TGFβ-1), platelet-derived growth factor (PDGF), and insulin-like growth factor-1 (IGF-1), are contained within the α-granules of platelets [26–29]. Within 10 minutes after PRP injection, the platelets aggregate and clot at the targeted site with almost 95% of the α-granules load being secreted within 1 hour [21]. Studies have shown that these growth factors are effective in promoting proliferation, angiogenesis, and synthesis of extracellular matrix proteins [26, 30–32].

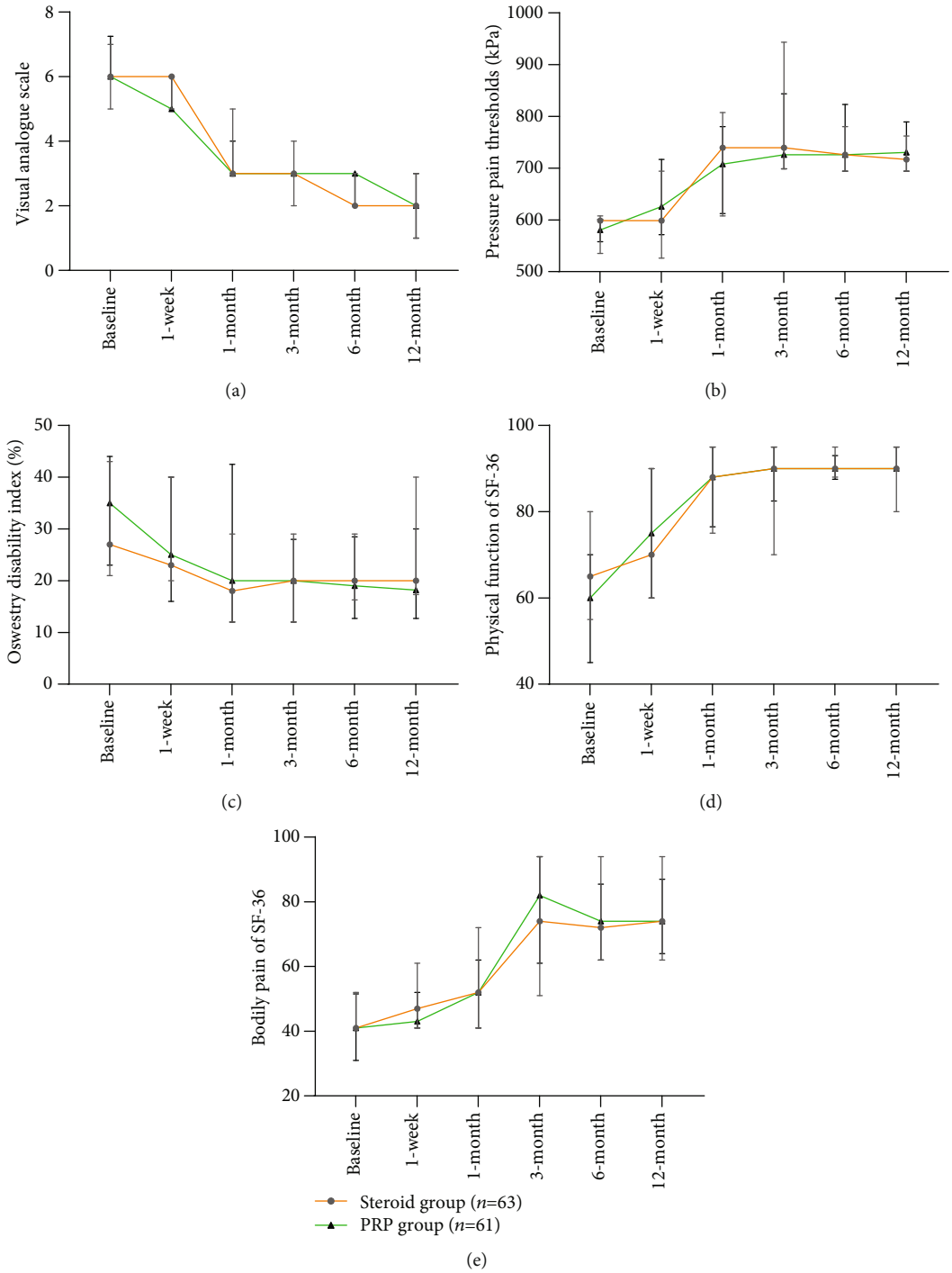

Figure 3: Comparison of patient-reported outcomes between the PRP ($n = 61$) and steroid ($n = 63$) groups over time (median, quartile). There was no significant difference in the (a) visual analogue scale, (b) pressure pain thresholds, (c) Oswestry disability index, and (d) physical function and (e) bodily pain domains of the 36-item short form health survey (SF-36) between the PRP group and the steroid group during follow-ups over the course of one year. The error bars represent the 1st quartile and the 3rd quartile.

Therefore, the key rationale behind the application of PRP is to increase the concentration of platelets at the targeted sites so that cytokines and GFs may be released. This will consequently allow the regulation of inflammation and immunological responses of tissue healing [21, 33].

In this investigation, sonography was used to guide transforaminal PRP and steroid injection. The outcomes of nerve root block under the guidance of sonography have proven to be similar to those of injections being guided by either computed tomography scan or X-ray [34–36]. Furthermore, sonography provides the advantages of real-time and dynamic observation with high accuracy, safe, convenient, no radiation, and avoidance of nerve or vessel injury.

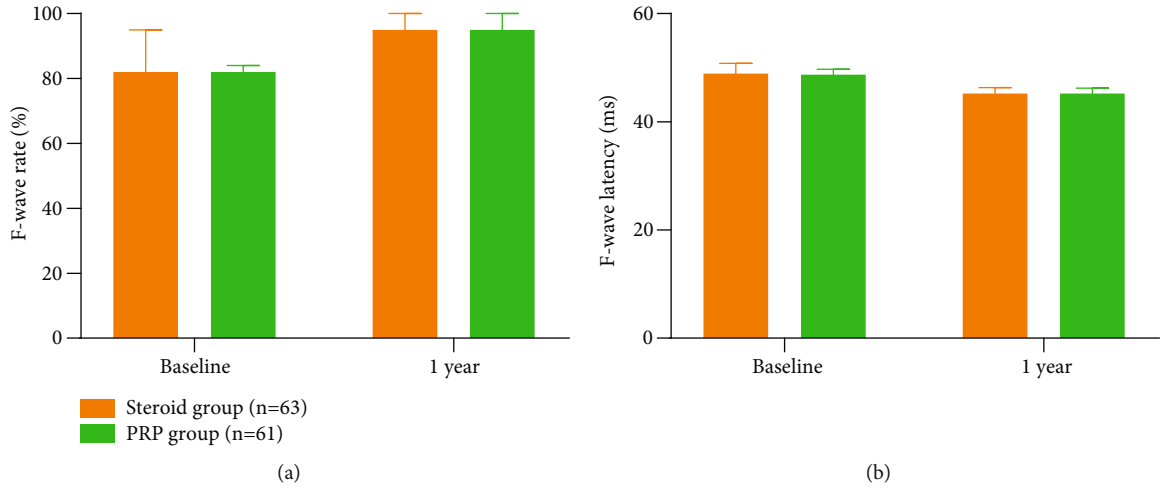

Figure 4: Comparison of F-wave rate and latency between the PRP ($n = 61$) and steroid ($n = 63$) groups. No significant difference was found in terms of F-wave (a) rate and (b) latency between the PRP group and the steroid group both before and after operation. The error bars represent the 3rd quartile.

No major complications and side effects were reported during the 1-year follow-up period in this study. Only one study reported very rare ischemic complications with lumbar epidural steroid injection by the interlaminar route [37]. Meanwhile, hematomas and infection are known to be the main complications of epidural steroid injection [38, 39]. Henceforth, this makes the autologous PRP a possibly safer alternative with low risks of infection and allergy, since PRP is derived from the patient's own blood and due to the presence of antibacterial proteins in platelets [40]. Furthermore, the systemic side effects of steroid can also be avoided with transforaminal PRP injection [41–43].

Nonetheless, there is currently a lack of standard procedure in PRP production for PRP therapy although a vast variety of formulations and techniques for PRP production is available. Other than that, the cost-effectiveness of PRP treatment remains controversial. On the one hand, the cost of PRP in Europe is reported to be about twice as much as the cost of steroid treatment [44]; on the other hand, a study reported that the cost of PRP therapy for treating orthopedic conditions may actually be less in the long run albeit it appearing to be more expensive than steroid injection in the short term [45].

This study was designed to focus on the long-term treatment effect of transforaminal injection of steroid or platelet-rich plasma (PRP) on lumbar disc herniation. Thus, the evaluation time points were mainly set at 1 month, 3 months, 6 months, and 1 year. The limitation of this study was the lack of short-term evaluation time point, including 2 weeks and 3 weeks. It could be further explored in future clinical trial.

5. Conclusions

This study suggests that ultrasound-guided transforaminal PRP injections yield similar effect as transforaminal steroid injections in treating lumbar disc herniation with radicular pain and that it may be a safer alternative in comparison.

Abbreviations

PRP: Platelet-rich plasma
PPTs: Pressure pain thresholds
VAS: Visual analogue scale
ODI: Oswestry disability index
PF: Physical function
BP: Bodily pain
SF-36: 36-item short form health survey
GFs: Growth factors
NRS: Numerical rating scale
VEGF: Vascular endothelial growth factor
TGFβ-1: Transforming growth factor β-1
PDGF: Platelet-derived growth factor
IGF-1: Insulin-like growth factor-1.

Data Availability

Participant original data used to support the findings of this study are available in http://www.medresman.org.cn/pub/cn/proj/search.aspx.

Ethical Approval

This study was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital, and the trial was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR-INR-17011825). This investigation was conducted in accordance with the Declaration of Helsinki.

Disclosure

The sponsors did not participate in the design, participant recruitment, data collections, analysis, and preparation of the paper.

Conflicts of Interest

The authors report no conflicts of interest in this work.

Authors' Contributions

Zhen Xu and Shaoling Wu contributed equally to this work and are the co-first authors.

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Supplementary Materials

The supplementary material was the CONSORT 2010 checklist of information to include when reporting a randomized trial. *(Supplementary Materials)*

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Research Article

The Wnt/β-Catenin Pathway Regulated Cytokines for Pathological Neuropathic Pain in Chronic Compression of Dorsal Root Ganglion Model

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Neuropathic pain is one of the important challenges in the clinic. Although a lot of research has been done on neuropathic pain (NP), the molecular mechanism is still elusive. We aimed to investigate whether the Wnt/β-catenin pathway was involved in NP caused by sustaining dorsal root ganglion (DRG) compression with the chronic compression of dorsal root ganglion model (CCD). Our RNA sequencing results showed that several genes related to the Wnt pathway have changed in DRG and spinal cord dorsal horn (SCDH) after CCD surgery. Therefore, we detected the activation of the Wnt/β-catenin pathway in DRG and SCDH and found active β-catenin significantly upregulated in DRG and SCDH 1 day after CCD surgery and peaked on days 7-14. Immunofluorescence results also confirmed nuclear translocalization of active β-catenin in DRG and SCDH. Additionally, rats had obvious mechanical induced pain after CCD surgery and the pain was significantly alleviated after the application of the Wnt/β-catenin pathway inhibitor XAV939. Furthermore, we found that the levels of proinflammatory factors tumor necrosis factor-α (TNF-α) and interleukin-18 (IL-18) were significantly elevated in CCD rat serum, while the levels of them were correspondingly decreased after the Wnt/β-catenin pathway being inhibited. The results of Spearman correlation coefficient analysis showed that the levels of TNF-α and IL-18 were negatively correlated with the mechanical withdrawal thresholds (MWT) after CCD surgery. Collectively, our findings suggest that the Wnt/β-catenin pathway plays a critical role in the pathogenesis of NP and may be an effective target for the treatment of NP.

1. Introduction

Low back pain (LBP) is the most common of all chronic pain disorders, and 80% of people experience LBP in their lifetime. LBP is often caused by lumbar foraminal stenosis (LFS) and lumbar disc herniation (LDH), causing symptoms such as motor dysfunction and NP [1]. In most parts of the world, LBP has become the main reason for limited mobility and

job loss, which brings a huge economic burden to individuals, families, and society [2, 3]. Despite decades of investigation and tremendous research effort in NP, the specific cellular and molecular mechanisms remain elusive. From previous studies, we know many processes participated in the production and persistence of NP, including hyperexcitability of neurons due to changes in ion channels on neuronal membrane such as sodium channels [4], calcium channels [5], potassium channels [6, 7], inflammatory factor accumulation [8, 9], and activation of glial cells [10–12].

The Wnt/β-catenin pathway is one of three Wnt pathways, and active β-catenin is the key protein on the Wnt/β-catenin pathway. In the absence of Wnts, the destruction complex which is located in the cytoplasm will bind active β-catenin and phosphorylate it to inactive β-catenin and after that proteasome will degrade β-catenin. While Wnts bind Frizzled (Fzd) and LRP5/6 on the cell membrane, the destruction complex will subsequently disintegrate, and then, active β-catenin is not phosphorylated and accumulates in the cytoplasm, subsequently translocating into the nucleus, in which active β-catenin interacts with T cell/lymphoid enhancer factor (TCF/LEF) transcription factors, resulting in the target gene transcription such as TNF-α and IL-18 [13–15]. Recent studies have found that conditional deletion of one copy of Wntless or β-catenin through Nestin-Cre-mediated recombination was sufficient to suppress the expression of brain-derived neurotrophic factor (BDNF), a major neurotrophic factor in mammals [16, 17]. Besides, several studies revealed the Wnt pathway plays an important role in the production and persistence of NP, such as partial sciatic nerve ligation (PSL) [18], spinal nerve ligation (SNL) [19], and chronic constriction injury (CCI) [15]. However, the role of this pathway has not been reported in the CCD model, which is the ideal model for studying this pain because it is a realistic model that mimics the pathological changes and clinical symptoms of LBP [20]. Based on the above research basis, we put forward a hypothesis about the Wnt/β-catenin pathway may make an important impact on the pathogenesis of NP induced by CCD.

In our research, we first found that several genes related to the Wnt pathway have undergone significant changes through RNA sequencing analysis, and then, we investigated the role of the Wnt/β-catenin pathway in the generation and persistence of NP using the CCD model which is a well-characterized rat model. The CCD model is divided into a single nerve root compression model and two nerve roots' compression model. In this study, we chose the L4 and L5 nerve roots' compression model, because clinically, most of the LFS and LBP produce compression on multiple nerve roots instead of a single nerve root [21], so L4 and L5 nerve roots' compression can better simulate the actual clinical situation. We first studied the activation of the Wnt/β-catenin pathway over time in DRG and SCDH after CCD-induced NP. After that, we inhibited the activation of the Wnt/β-catenin pathway in DRG and SCDH through using XAV939, a small molecule tankyrase inhibitor which targets the Wnt/β-catenin pathway and inhibits the abnormal activation of the Wnt/β-catenin pathway without affecting normal function of cells [22], to observe if CCD-induced noxious hypersensitivity can be alleviated. XAV939 has good plasma stability and can be administered by intraperitoneal injection, which is more convenient than other Wnt pathway inhibitors [23]. Finally, we explored the potential mechanism of Wnt/β-catenin signaling in the generation and persistence of NP.

2. Experimental Procedures

2.1. Animals. Fifty-nine SD (Sprague Dawley) male rats (220 g-250 g body weight) were purchased from Shanghai Jiesijie Experimental Animal Co., Ltd (Shanghai, China, license no. SCXK (Hu) 2018-0004). Animals were housed in padded cages at a constant temperature (12 : 12 h light-dark cycle) and got food irradiated by the standard laboratory (Shanghai Jiesijie Experimental Animal Co., Ltd, China) and tap water with freedom. The animal experiments and handlings were approved by the Animal Ethics Committee of Tongji Hospital, Shanghai.

2.2. CCD Surgery. To produce intervertebral foramen stenosis and NP, the CCD model was used. The procedure for CCD has been described [24], and the schematic diagram of CCD is shown in Figure 1(a). Briefly, under 1% sodium pentobarbital anesthesia (0.4 ml/kg, i.p.) and sterile conditions, the left L4 and L5 intervertebral foramina of the rat were exposed, and then, two L-shaped stainless steel rods with a diameter of 0.6 mm and a length of 4 mm were inserted into the two intervertebral foramina, respectively, to produce chronic compression on the DRG. Finally, rats were kept in a single plastic cage after suturing muscle and skin. As for the sham group, the procedure is the same as that of the CCD group above but without the insertion of stainless steel rods [24]. During the experiment, no death or self-harm occurred in the rats. On the first postoperative day, rats with insignificant pain behavior were excluded (no positive response at 6 g).

2.3. Pharmacological Inhibition of the Wnt/β-Catenin Pathway. XAV939, an inhibitor of the Wnt/β-catenin pathway (Abmole, USA), in 10% DMSO/90% 0.9% NaCl, was injected in doses of 1.25 mg/kg intraperitoneal. Besides, the sham+vehicle group and the CCD+vehicle group also received an injection (200 μl of 10% DMSO/90% 0.9% NaCl). The injections above were given at about 10 am every day for seven consecutive days.

2.4. Assessment of MWT. The rats were placed in a transparent box on the test grid at 8 am on test day, maintained at 25°C at room temperature, and adapted to the environment for 15-30 min. Von Frey hairs (Stoelting, USA) were applied, according to former reports [25], and the Von Frey hair number was selected as described [26], in brief, starting from 0.6 g, gradually increasing 1.0 g, 1.4 g, 2.0 g, 4.0 g, 6.0 g, 8.0 g, 10.0 g, 15.0 g, and 26.0 g. The sole of the hind paw was the site of stimulation of Von Frey hair, and the testing was performed only on the ipsilateral side. If the response was positive, the adjacent decreasing Von Frey hair was selected for the next stimulation; if the response was negative, the adjacent increasing hair was selected. The assessment was over

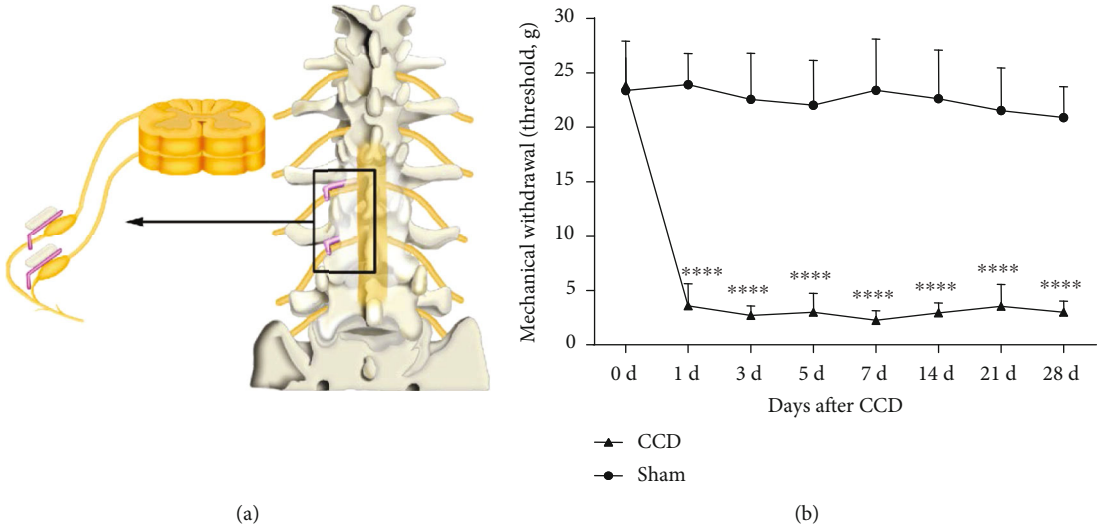


Figure 1: The CCD model and the development of mechanical allodynia after establishment of the CCD. (a) Schematic diagram of CCD generated by inserting stainless steel rod into L4 and L5 intervertebral foramina of rats. (b) CCD-induced mechanical allodynia is manifested by a reduction of MWT. Bars represent group means ± SD, $n = 12$ per group. Two-way ANOVA, $^{****}P < 0.0001$ versus sham.

when the maximum number of grams of 26.0 g was used and the response was still negative, or the test was performed three times after the first positive reaction. The MWT was calculated by the analyzer who was blind to the group data.

2.5. RNA Sequencing and Differentially Expressed Gene (DEG) Screening. Rats were anesthetized with 2% pentobarbital sodium (80 mg/kg) by intraperitoneal injection 28 days after CCD and decapitated after reaching full anesthesia. The left L4 and L5 nerves were quickly exposed and were carefully extracted. Then, the enlarged DRG was cut with a blade on ice. Later, according to the positioning of the lumbar enlargement, the dorsal horn of the left spinal cord of L4-5 segment was removed. All the tissues above were, respectively, placed in centrifuge tubes containing RNA later (QIAGEN, Valencia, CA) and were subsequently transferred to a 4°C refrigerator. RNA sequencing was completed by Shanghai Genergy Biotechnology Company, and the general protocol was as follows: 1 μl RNA was taken for quantification and 500 ng RNA was taken for library construction according to the quantification results. After that, the library was subjected to quality inspection using Qubit instruments (Invitrogen, USA). Then, Illumina HiSeq was used for sequencing, and the instrument was NovaSeq 6000 (Illumina, USA). Deseq2 software (v1.16.1) was used to screen for DEGs, and the screening criteria are P value ≤ 0.05 and $|\log_2 \text{fold change}| \geq 1$.

2.6. Western Blot Analysis. Rats were anesthetized with 2% pentobarbital sodium (80 mg/kg) by intraperitoneal injection and decapitated after reaching full anesthesia. The left L4-L5 DRG and SCDH segments were homogenized, respectively, by using the RIPA lysis buffer and then protein samples were quantified by applying BCA Protein Assay (Beyotime Biotechnology, Shanghai, China). The protein samples were separated by 10% SDS-PAGE (Beyotime Biotechnology, Shanghai, China) after being heated for 5 min at 99°C and subsequently transferred to 0.45 μm PVDF membrane (Millipore, Billerica, MA). The membranes were treated with primary antibodies overnight at 4°C, after being blocked with 5% skimmed milk diluted in TBST, followed by being incubated in appropriate secondary antibodies. The following primary antibodies were used: rabbit anti-active β-catenin (1 : 1000 dilution; CST, Danvers, MA, USA), mouse anti-β-tubulin (1 : 1000 dilution; Sigma-Aldrich, Darmstadt, Germany), and horseradish peroxidase-linked secondary anti-rabbit or anti-mouse antibodies (1 : 1000 dilution; Beyotime Biotechnology, Shanghai, China). Finally, the blots were visualized by DRAFT-FluorChem Q (Alpha Innotech Corporation, San Leandro, CA, USA) and optical density of all bands was conducted by the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.7. Immunohistochemistry. Rats were anesthetized with 1% pentobarbital sodium (40 mg/kg) by intraperitoneal injection. 0.9% saline and 4% paraformaldehyde were sequentially administered to these anesthetized rats. The L4 and L5 DRG were removed intact and subsequently fixed in 4% formaldehyde overnight. These tissues were then dehydrated with gradient sucrose (10%, 20%, and 30%) and frozen with OCT as they settled to the bottom in the 30% sucrose solution. After the embedding was completed, the tissues were sliced, and the thicknesses of the DRG and spinal cord sections were 10 μm and 15 μm, respectively. Next, immunofluorescence staining was performed, and the steps are as follows: after being blocked with a 0.01 M PBS blocking solution containing 10% goat serum and 0.3% Triton X-100 for 1 h at 37°C, the sections were incubated with the primary antibody overnight at 4°C (rabbit anti-active β-catenin, 1 : 800, Millipore), followed by being incubated in goat anti-rabbit Cy3-conjugated secondary antibody (1 : 300 dilution, Jackson ImmunoResearch, Amish, PA) for 2 hours at 37°C in the dark. Subsequently, sections were stained with nuclear dye DAPI (1 : 1000 dilution, Invitrogen, Carlsbad, CA, USA) for

Table 1: DEGs related to Wnt pathway in DRG and SCDH of rats 28 days after CCD.

Gene symbol	Description	$\log_2$ fold change	Direction	P value
DRG				
Npy	Neuropeptide Y	3.9458	↑	<0.001
Runx2	Runt-related transcription factor 2	1.3276	↑	<0.001
Cthrc1	Collagen triple helix repeat containing 1	2.3058	↑	<0.0001
Draxin	Dorsal inhibitory axon guidance protein	2.3726	↑	<0.01
Cpz	Carboxypeptidase Z	-1.2635	↓	<0.01
Kb15	Type II keratin Kb15	-1.9539	↓	<0.05
Tnn	Tenascin N	2.6944	↑	<0.01
Amer2	APC membrane recruitment protein 2	-1.8238	↓	<0.01
Pcdh20	Protocadherin 20	1.7709	↑	<0.001
Pcdh8	Protocadherin 8	-1.1441	↓	<0.01
Myh3	Myosin heavy chain 3	-1.9875	↓	<0.05
Gnb3	G protein subunit beta 3	-1.0005	↓	<0.05
SCDH				
Mcc	Colorectal mutant cancer protein	-2.2131	↓	<0.05
Egf	Epidermal growth factor	1.1111	↑	<0.0001
Cdh1	Cadherin 1	1.7580	↑	<0.05
Plekha4	Pleckstrin homology domain containing A4	1.4888	↑	<0.001

7 minutes at 37°C in the dark. The sections were imaged with the confocal microscope (Nikon, A1 MP+, USA) and fluorescence microscope (Nikon, Ni-U, USA).

2.8. ELISA (Enzyme-Linked Immunosorbent Assay). The rats in the deep anesthetized with sodium pentobarbital (80 mg/kg) were subjected to blood collection from the orbital venous plexus, and then, blood collected was centrifugated to get serum at 4000 rpm for 10 min at 4°C. Detailed steps were performed according to the kit (WESTANG BIO-TECH, Shanghai, China) instructions. The optical density (OD) value was measured by DENLEY DRAGON Wellscan MK 3 (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm, and all OD values were calculated after subtracting the blank value. Ascent software for Multiskan (Thermo Fisher Scientific, MA, USA) was applied to draw a standard curve, and the corresponding TNF-α and IL-18 concentrations were calculated according to the OD value of the sample.

2.9. Statistical Analysis. GraphPad Prism 7.0 software (GraphPad Software Inc., CA, USA) and SPSS 20.0 software (IBM Corporation, NY, USA) were applied to analyze all data. The number (N) of rats and the statistical significance were stated in the figures and figure legends. The sample size was determined based on previous experience. Two-way ANOVA was used to analyze the result of behavior, and one-way ANOVA was applied to analyze the western blot and ELISA. Spearman correlation coefficient analysis was used to analyze the correlation between MWT and inflammatory factor levels, including TNF-α and IL-18. Data are represented as mean ± SD (standard deviation). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Induction and Persistence of Allodynia in CCD Model Rats. To confirm that the CCD model successfully and reproducibly induced tactile allodynia (Figure 1(a)), we performed gait and posture observations and measurements of MWT in CCD rats. We found that rats had gait and posture abnormalities after surgery, such as the hind paw of the operation side curled up and did not dare to bear weight. We tested the MWT of rats at different time points. The results showed that continuous compression of DRG significantly reduced the MWT of the hind paw and was consistent with previous studies [27, 28], as shown in Figure 1(b). The MWT decreased significantly on the first day after CCD and reached the lowest point on the 7th day ($P < 0.0001$). By the 28th day after surgery, the MWT remained at a low level ($P < 0.0001$). The MWT at each time point in the CCD group was lower than that in the sham group ($P < 0.0001$), while no obvious allodynia was observed in the sham group ($P > 0.05$).

3.2. Changes in the Expression of Wnt-Related Genes of DRG and SCDH in CCD. For selection of a subset of genes related to the Wnt pathway, the NCBI gene database and the Panther Classification System database system were used. "Wnt pathway" was used as a keyword to conduct a restrictive search for "rattus norvegicus." 526 genes related to the Wnt pathway were obtained in the NCBI gene database. The overlapping genes of these data sets and the DEGs (differentially expressed genes) screened in the DRG and SCDH were analyzed using an online tool of the Venn diagram, and the results showed that 8 DEGs and 4 DEGs in DRG and SCDH were related to the Wnt pathway, respectively. Then, we performed gene ontology analysis by the Panther Classification

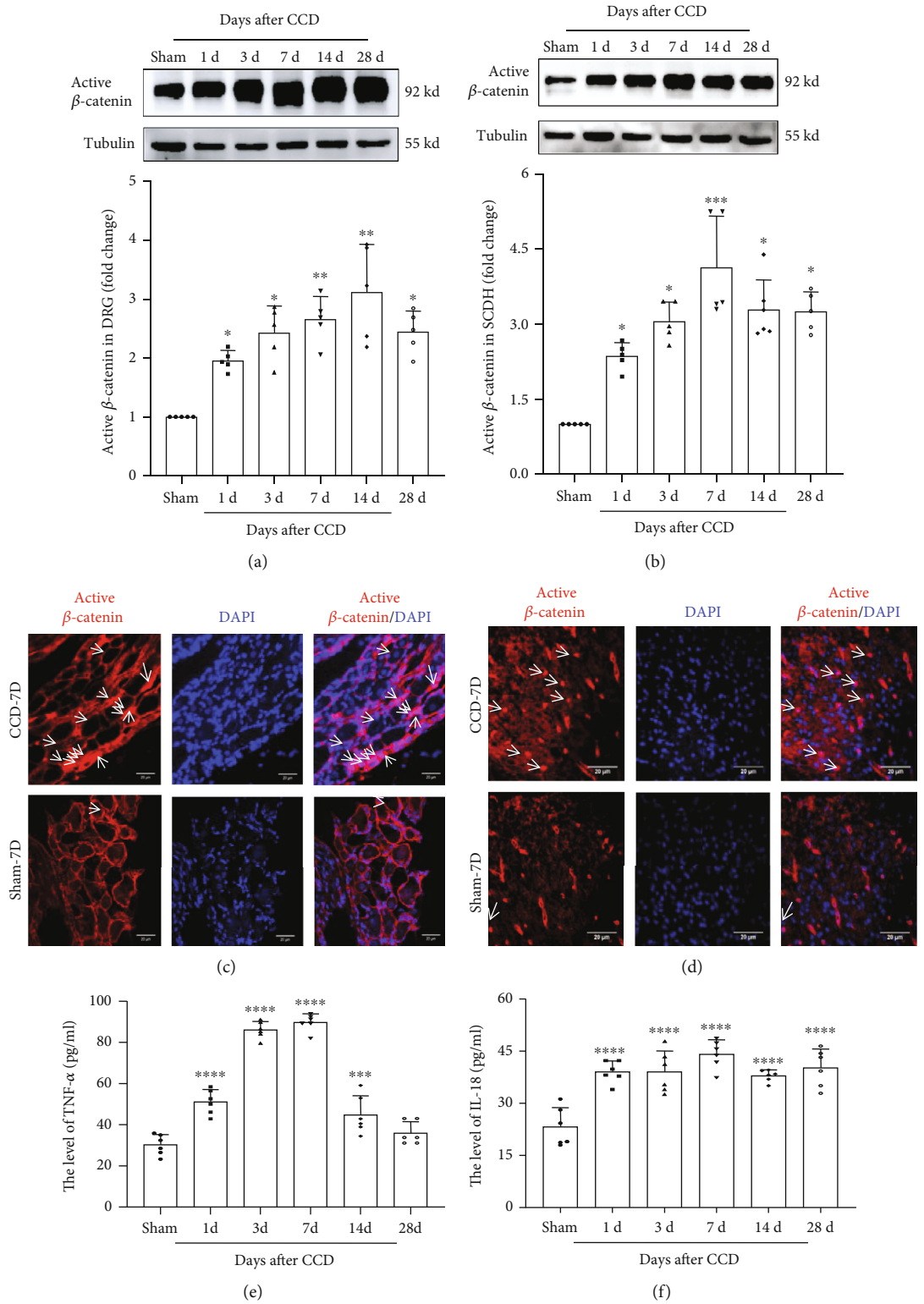
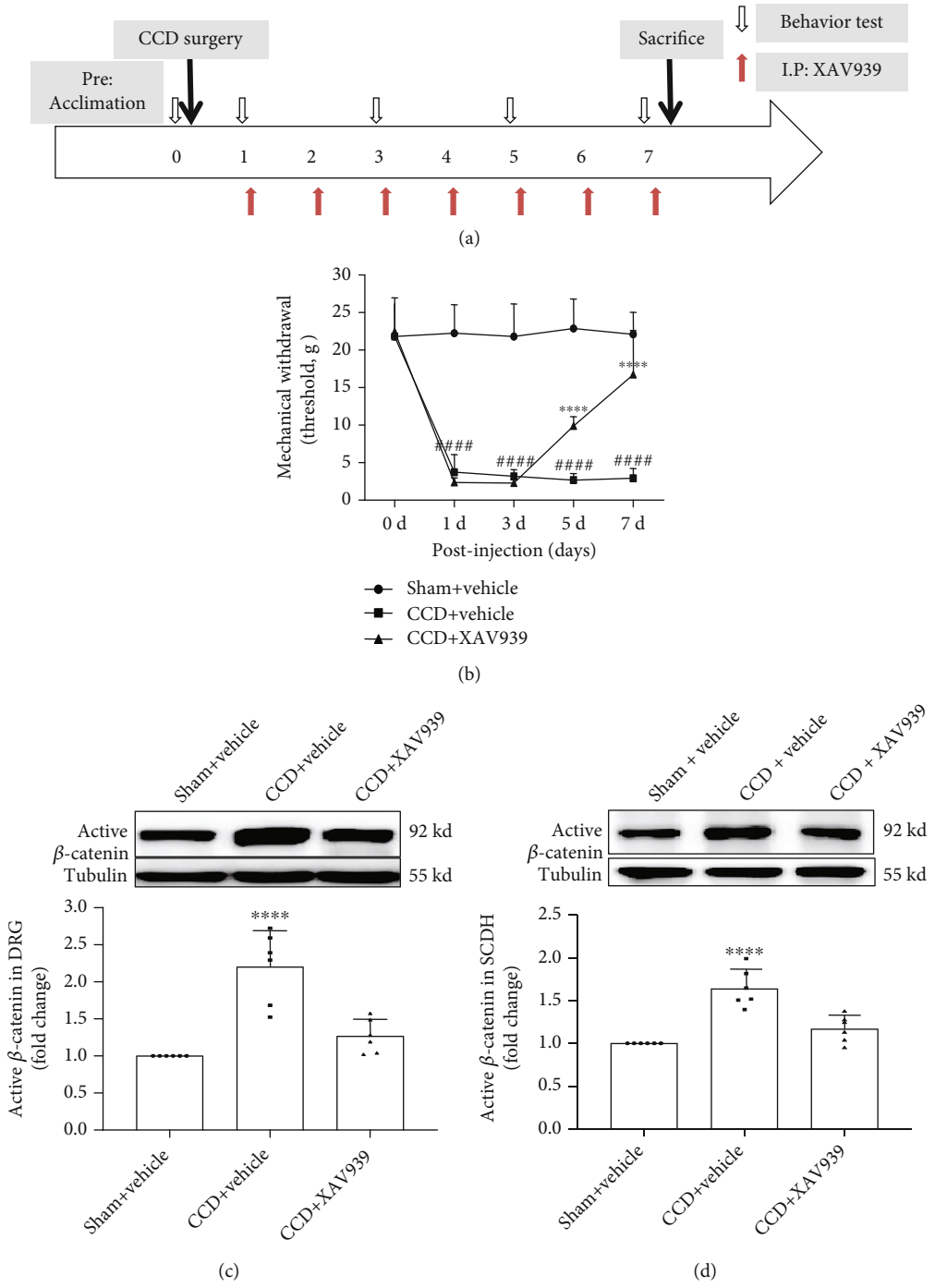


FIGURE 2: Expression and nuclear translocation of active β-catenin protein in DRG and SCDH and the levels of TNF-α and IL-18 in serum after CCD. Western blot showing the expression of active β-catenin in (a) DRG and (b) SCDH at different time points. The expression of active β-catenin (red) and its transport of the superficial (c) DRG and (d) SCDH into the nucleus (DAPI). Arrows indicate some of the nuclear transport of active β-catenin. Tissues were collected on CCD day 7. The sections were imaged with a (c) fluorescence microscope and a (d) confocal microscope. Original magnification, scale bars: 20 μm. ELISA displaying the levels of (e) TNF-α and (f) IL-18 in rat serum at different time points after CCD ($n = 6$). Serum was collected on CCD day 7. Bars represent group means ± SD, $n = 5$. One-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ versus sham.



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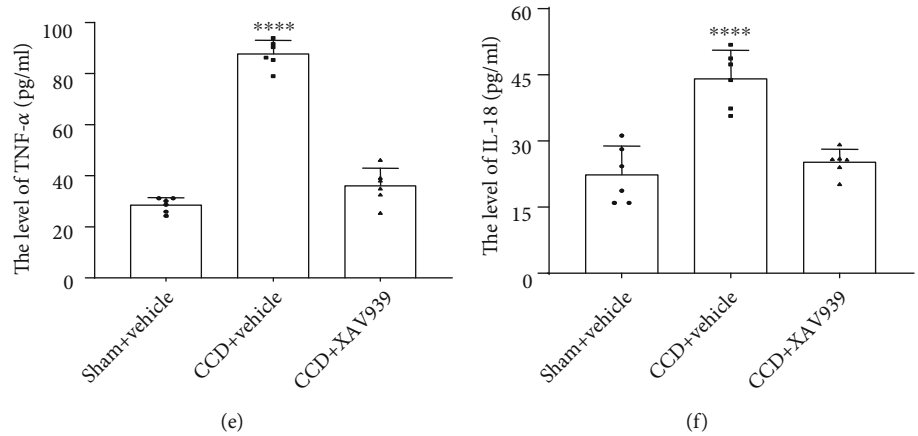

Figure 3: Intraperitoneal injection of XAV939 attenuated mechanical allodynia and decreased TNF-α and IL-18 levels in serum after CCD. (a) Schematic time course for acclimation, CCD surgery, intraperitoneal injection of XAV939, behavioral testing, and sacrifice. (b) MWT measured on 0, 1, 3, 5, and 7 days after CCD treatment ($n = 9$). Two-way ANOVA. Western blot showing active β-catenin expression in (c) DRG and (d) SCDH ($n = 6$). ELISA showing the expression of (e) TNF-α and (f) IL-18 in the serum of rats after intraperitoneal injection of XAV939 ($n = 6$). Serum was collected at different time points after CCD. One-way ANOVA. Bars represent group means ± SD. $^{\#\#\#\#}P < 0.0001$, CCD+vehicle versus sham; $^{****}P < 0.0001$, CCD+XAV939 versus CCD+vehicle.

System database system to enrich the pathways involved in proteins encoded by DEGs in DRG and SCDH and found that 4 DEGs in DRG and 1 DEG in SCDH related to the Wnt pathway were screened out. After removing the duplicated genes, we screened out 12 and 4 genes in DRG and SCDH, respectively (Table 1).

3.3. Activation of Wnt/β-Catenin Induced Cytokines in Both DRG and SCDH in CCD. DRG and SCDH changes caused by nerve injury are critical for the generation and persistence of NP. We addressed whether the Wnt/β-catenin pathway in DRG and SCDH in CCD rats has changed. Active β-catenin is a critical protein in this pathway and whether it undergoes nuclear translocation reflects whether Wnt/β-catenin is activated. Therefore, we detected the level of active β-catenin by western blot and nuclear translocation of active β-catenin by immunofluorescence. As is shown in western blot results, the compression of DRG resulted in a rapid rise in the expression of active β-catenin in DRG (Figure 2(a)) and SCDH (Figure 2(b)) (within 1 day) and increased continuously ($P < 0.05$). Both reached a peak of expression in 7-14 days and then gradually declined. Additionally, immunofluorescence experiments showed that after 7 days of modeling, a large number of active β-catenin positive stains (red) were seen on the left DRG (Figure 2(c)) and SCDH (Figure 2(d)) in the CCD model rats, and the positive staining was stronger than that in the sham group. Colocalization of active β-catenin (red) and DAPI (blue) showed that the expression of active β-catenin in the nucleus rose in CCD rats. To further explore the potential mechanism of the Wnt/β-catenin pathway affecting NP, we detected the expression levels of TNF-α and IL-18, which are the proinflammatory cytokines in serum by ELISA. The results showed that the level of TNF-α and IL-18 in rat serum increased significantly on the first day ($P < 0.0001$) and reached the peak at 7 days, and then, TNF-α gradually decreased, while IL-18 remained at a stable level until postoperative 28 days ($P < 0.0001$) (Figures 2(e) and 2(f)). In addition, the results of Spearman correlation coefficient analysis showed that the levels of TNF-α (Spearman correlation coefficient: -0.829, $P < 0.05$) and IL-18 (Spearman correlation coefficient: -0.943, $P < 0.01$) were negatively correlated with MWT after CCD surgery, which meant that the higher the levels of TNF-α and IL-18, the more obvious the pain in rats, and vice versa, the pain in rats was relieved.

3.4. Blocking the Wnt/β-Catenin Pathway Suppressed the Cytokines for the Development of Allodynia in CCD. Next, to investigate whether the Wnt/β-catenin pathway makes a critical impact on the pathogenesis and maintenance of neuropathology, we performed intraperitoneal injection of XAV939, a Wnt/β-catenin pathway inhibitor, in CCD rats for seven consecutive days and tested behavioral changes daily (timeline is shown in Figure 3(a)). Our results showed that XAV939 can alleviate NP after CCD. At 5-7 days of injection, the MWT of the hind paw significantly increased ($P < 0.0001$) (Figure 3(b)). Furthermore, we confirmed that XAV939 did inhibit the Wnt/β-catenin pathway via western blot and immunofluorescence experiments. The results showed that XAV939 did inhibit active β-catenin accumulation in DRG and SCDH (Figures 3(c) and 3(d)). Correspondingly, the expression of TNF-α and IL-18 was also significantly reduced (Figures 3(e) and 3(f)) when we inhibited this pathway by XAV939 ($P < 0.0001$). The results above demonstrated that the activation of the Wnt/β-catenin pathway affected the persistence of NP and the expression of TNF-α and IL-18 was regulated by the activation of the Wnt/β-catenin pathway after CCD.

4. Discussion

LBP is one of the most common pains in the clinic. Mechanical compression and chemical stimulation of DRG may lead to ischemia, edema, and degeneration of DRG and eventually

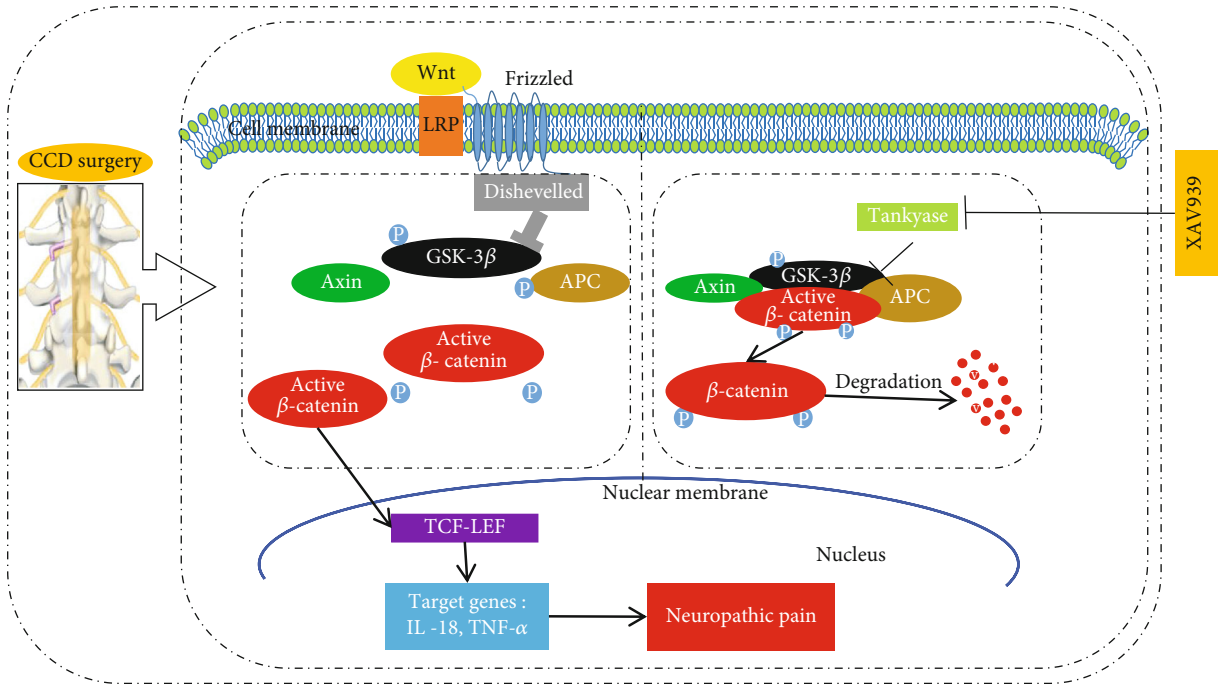

Figure 4: Schematic illustration of the relationship between the Wnt/β-catenin pathway and neuropathic pain. The relationship between the Wnt/β-catenin pathway and neuropathic pain in the CCD model was possibly that CCD surgery caused activation of the Wnt/β-catenin pathway; then, active β-catenin accumulated in the cytoplasm and translocated into the nucleus. Subsequently, the transcription of the target genes TNF-α and IL-18 increased, leading to neuropathic pain finally, while the Wnt/β-catenin inhibitor XAV939 can suppress this process, relieving CCD-induced neuropathic pain.

leading to the development of allodynia [29, 30]. However, the specific mechanism of NP is still unclear. Our study brings a key role for the Wnt/β-catenin pathway in the induction and continuation of NP following chronic compression of nerve to light. The main findings can be attributed to the following three aspects: (1) chronic nerve compression led to changes in the expression of multiple Wnt pathway-related genes and rapid and sustained activation of the Wnt/β-catenin pathway in DRG and SCDH; (2) inhibition of activation of the Wnt/β-catenin pathway alleviated allodynia induced by chronic nerve compression; (3) CCD led to elevating the level of TNF-α and IL-18. TNF-α and IL-18 levels were significantly correlated with MWT, while blocking the Wnt/β-catenin pathway decreased the levels of TNF-α and IL-18.

Wnt is very important for various development processes. The Wnt pathway was first revealed by scientists in the development of Drosophila [31] and is a key signaling pathway regulating neuronal development, neuronal polarization, synaptic plasticity changes, and directional growth of axons and dendrites [32, 33]. Previous studies have revealed that the Wnt/β-catenin pathway is activated in SCDH in several NP models, such as CCI and PSL [15, 18]. Besides, studies showed that the levels of multiple inflammatory factors increased in the serum of clinical patients including IL-18 and TNF-α [34, 35] and pain intensity correlated with the levels of inflammatory factors [36]. The reason for choosing TNF-α and IL-18 among many elevated inflammatory factors was that these two inflammatory factors were not only closely related to pain, but also target genes closely downstream of the Wnt/β-catenin pathway [15]. These studies suggested that the Wnt/β-catenin pathway makes an important impact on the development and progression of NP, and the activation of this pathway may participate in the pathogenesis of NP by regulating the transcription of TNF-α and IL-18, which are closely related to pain.

Our study found that active β-catenin was immediately and continuously upregulated in DRG and SCDH in the CCD model, and immunofluorescence results confirmed the translocation of active β-catenin into the nucleus, which can more intuitively observe the localization of active β-catenin protein in cells and more powerful than the extraction of active β-catenin protein in the nucleus to prove the nuclear translocation of active β-catenin protein and the activation of the Wnt/β-catenin pathway. This suggested the activation of the Wnt/β-catenin pathway lasted for a long time, which was consistent with previous studies in other models [15, 18]. Additionally, we found that the levels of TNF-α and IL-18, which are closely related to NP, were considerably raised in the serum of CCD rats; however, after inhibiting this pathway, the levels of TNF-α and IL-18 correspondingly decreased. Moreover, the levels of TNF-α and IL-18 were significantly correlated with the allodynia time of CCD rats. These results implicated blocking the Wnt/β-catenin pathway can inhibit NP by decreasing the level of IL-18 and TNF-α. These results revealed that the Wnt/β-catenin pathway takes part in the development of NP after compression of DRG and is likely to affect NP by regulating the transcription of downstream inflammatory factors. Mechanisms underlying contributions of the Wnt pathway to neuropathic pain are summarized in Figure 4.

What is more, many scholars have conducted extensive research on the mechanism of NP, and it is believed that NP is caused by peripheral sensitization and central sensitization [37, 38]. Peripheral sensitization refers to an increase in the excitatory persistent abnormalities of primary afferent neurons, resulting in increased pain signal production [39]; central sensitization refers to the plasticity change of synaptic in SCDH, and the long-term potential (LTP) of synaptic transmission efficiency in the pain transmission pathway [40]. Studies have shown that the Wnt pathway can regulate neuronal firing activity [41, 42] and enhance synaptic plasticity by promoting synapse formation such as in the hippocampus [43, 44]. Therefore, we conjectured that the Wnt/β-catenin pathway may also induce peripheral sensitization and central sensitization by increasing neuronal excitability in DRG and increasing synaptic strength in SCDH, thereby affecting the progression of NP.

In summary, our research brings a pivotal role for the Wnt/β-catenin pathway in the production and persistence of NP after chronic compression of DRG to light. Additionally, the Wnt/β-catenin pathway may induce NP through modulating the levels of TNF-α and IL-18. Moreover, it provides new targets and new ideas for the treatment of nerve compression diseases including LFS and LDH and may also be one of the ways for some drugs and biological treatments such as stem cells to exert analgesic effect.

Data Availability

GraphPad Prism 7.0 software (GraphPad Software Inc., CA, USA) and SPSS 20.0 software (IBM Corporation, NY, USA) were applied to analyze all data.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

YZ, YHM, XMC, and DSX designed the experiments and prepared the figures. YZ and DZ performed all the experiments. YZ, DZ, XMC, and DSX analyzed the data. All authors contributed to writing and approved the manuscript for submission. Ye Zhang is the first author, Dongsheng Xu is the first co-corresponding author, Yanhong Ma and Xuemei Chen are the third and second co-corresponding authors.

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Research Article

LIFU Alleviates Neuropathic Pain by Improving the KCC_2 Expression and Inhibiting the CaMKIV–KCC_2 Pathway in the L4–L5 Section of the Spinal Cord

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Effective treatment remains lacking for neuropathic pain (NP), a type of intractable pain. Low-intensity focused ultrasound (LIFU), a noninvasive, cutting-edge neuromodulation technique, can effectively enhance inhibition of the central nervous system (CNS) and reduce neuronal excitability. We investigated the effect of LIFU on NP and on the expression of potassium chloride cotransporter 2 (KCC_2) in the spinal cords of rats with peripheral nerve injury (PNI) in the lumbar 4–lumbar 5 (L4–L5) section. In this study, rats received PNI surgery on their right lower legs followed by LIFU stimulation of the L4–L5 section of the spinal cord for 4 weeks, starting 3 days after surgery. We used the 50% paw withdraw threshold (PWT_{50}) to evaluate mechanical allodynia. Western blotting (WB) and immunofluorescence (IF) were used to calculate the expression of phosphorylated extracellular signal–regulated kinase 1/2 (p-ERK1/2), calcium/calmodulin-dependent protein kinase type IV (CaMKIV), phosphorylated cyclic adenosine monophosphate response element-binding protein (p-CREB), and KCC_2 in the L4–L5 portion of the spinal cord after the last behavioral tests. We found that PWT_{50} decreased ($P < 0.05$) 3 days post-PNI surgery in the $LIFU^-$ and $LIFU^+$ groups and increased ($P < 0.05$) after 4 weeks of LIFU stimulation. The expression of p-CREB and CaMKIV decreased ($P < 0.05$) and that of KCC_2 increased ($P < 0.05$) after 4 weeks of LIFU stimulation, but that of p-ERK1/2 ($P > 0.05$) was unaffected. Our study showed that LIFU could effectively alleviate NP behavior in rats with PNI by increasing the expression of KCC_2 on spinal dorsal corner neurons. A possible explanation is that LIFU could inhibit the activation of the CaMKIV–KCC_2 pathway.

1. Introduction

Neuropathic pain (NP) is defined as pain originating from primary lesions and dysfunction of the somatosensory system, either at the peripheral or central level [1]. Many studies have been conducted on this type of pain, and some progress has been made, but many challenges remain in the clinical treatment of NP [2]. The main clinical manifestations include spontaneous pain, persistent (or paroxysmal) pain, induced pain, paresthesia, numbness, and tingling [2, 3]. As a refractory and chronic pain that can manifest in various ways, including as chronic low back pain or sciatica, NP is a severe problem. It affects 6.9%–10% of the population worldwide and seriously diminishes patient's quality of life [2], increasing the economic burden on the patient's family and on society [4–6].

The etiology and mechanism of NP are complicated and unclear. Recently, an increasing amount of evidence has shown that downregulation of potassium chloride cotransporter 2 (KCC_2) in the spinal cord plays an important role in NP. KCC_2 is an ion transporter protein present in mature neurons of the central nervous system (CNS) and can remove Cl^- from the cytoplasm to the extracellular space [7]. After peripheral nerve injury (PNI), the expression of KCC_2 on the neuronal membrane is downregulated, and the concentration of Cl^- ($[Cl^-]_i$) in nerve cells is upregulated, thereby reducing the inhibitory effect of the neurotransmitter γ-aminobutyric acid (GABA) [8–10]. GABA, the main inhibitory neurotransmitter in the mature CNS [11], can bind to the GABA receptor (GABA-R) to promote depolarization of the postsynaptic nerve membrane and mediate hyperpolarization and activity of the neuron [12]. Dysfunction of GABA-R

eventually reduces inhibition of the spinal cord, leading to hyperexcitability of primary afferent neurons and activation by low-threshold mechanical sensory input. Simultaneously, the primary afferent neurons respond only to high-threshold (nociceptive) inputs under normal circumstances, thereby causing mechanical allodynia and NP [13, 14]. NP has been successfully induced in rats by injecting microribonucleic acid (miRNA), which interferes with the transcription of KCC_2, or a KCC_2 inhibitor [15, 16]. All results suggest that downregulation of KCC_2 after PNI plays an essential role in the development of NP [17]. Many studies have found that increasing the expression of KCC_2 significantly relieves NP behavior [9, 18–20]. Therefore, learning how to increase the expression of KCC_2 following PNI has great potential value for treating NP.

After PNI, nociceptive stimulation leads to downregulation of the KCC_2 expression through a series of intracellular cascades in the brain-derived neurotrophic factor- (BDNF-) tropomyosin receptor kinase B (TrkB) pathway [21, 22]. Calcium/calmodulin-dependent protein kinase type IV (CaMKIV), phosphorylated cyclic adenosine monophosphate response element-binding protein (p-CREB), and phosphorylated extracellular signal–regulated kinase 1/2 (p-ERK1/2) play essential roles in activation of the BDNF–TrkB pathway cascades and downregulation of the KCC_2 expression [10, 23, 24]. Rivera confirmed in transgenic mice that activation of the TrkB receptor by BDNF further inhibited the expression of KCC_2 at the transcriptional level via the intracellular phosphoinositide phospholipase C gamma $(PLC\gamma) \longrightarrow Ca^{2+} \longrightarrow CaMKIV \longrightarrow p-CREB$ cascade [23]. Recently, studies also have found that nociception such as PNI or inflammation can activate the ERK–mitogen-activated protein kinase (MAPK) pathway in the spinal dorsal horn, upregulate the intracellular p-ERK1/2 expression via a Ras⟶p-ERK1/2 cascade reaction, and inhibit the expression of KCC_2 at the transcriptional level, ultimately leading to NP [23, 25, 26]. Therefore, the CaMKIV–KCC_2 or p-ERK1/2–KCC_2 pathway plays a vital role in NP pathogenesis after PNI (Figure 1).

At present, due to its complicated mechanism, there is still no satisfactory treatment for NP [27, 28]. At the clinical level, conventional painkillers such as tricyclic antidepressants, anticonvulsants, nonsteroidal anti-inflammatory drugs (NSAIDs), antiepileptic drugs, and weak and strong opioids are often used for symptomatic relief, but with poor efficacy and many side effects [28–30]. Therefore, finding a suitable rehabilitation method for NP would have great clinical significance. As a form of noninvasive neuromodulation, low-intensity focused ultrasound (LIFU) has been confirmed safe for modulating brain activity in patients and animals with seizures [31], Alzheimer's disease and dementia [32], traumatic brain injury (TBI) [33], and depression [34]. LIFU's neuromodulatory mechanism includes mechanical, thermal, and cavitation effects [35]. The mechanical effect of LIFU plays an important role in neuromodulation, and its mechanism might be that acoustic radiation forces the bimolecular structure of the cell membrane to stretch through mechanical vibration, thereby interfering with the mechanically sensitive ion channels on the cell membrane and producing the corresponding biological effect [36, 37]. Interestingly, King et al. applied LIFU to the CNS in epileptic rats and found that it could inhibit abnormal epileptic discharge by activating GABAergic neurons in the CNS [38].

However, whether spinal cord stimulation with LIFU can enhance the inhibitory effect and alleviate NP is still unclear. In this study, we loosely ligated the right tibial nerve and common peroneal nerve in rats to create a PNI model. After LIFU stimulation of the L4–L5 spinal cord section, we used the 50% paw withdraw threshold (PWT_{50}) to evaluate the rats' mechanical stimulation threshold; WB and IF were used to detect the expression changes of p-ERK1/2, CaMKIV, p-CREB, and KCC_2 in the lumbar spinal cord.

2. Materials and Methods

2.1. Animals. We acquired a total of 40 healthy male Sprague-Dawley (SD) rats (weight, 220–300 g) from Kunming Laboratory Animal Center (Kunming, China) for use in the experiment. All rats were housed at 25°C ± 2°C on a 12 h reverse light/dark cycle in separate cages (5 rats per cage) and had free access to food and water. All animal protocols were approved by the Animal Ethics Committee of Kunming Medical University (No. KMMU2020352).

2.2. Grouping and Experimental Design. After 1 week of adaptation, all rats were randomly divided into four groups (10 per group): normal group, rats that received neither surgery nor treatment; sham group, rats in which nerves were exposed according to the PNI surgical method but not ligated; and $LIFU^-$ group and $LIFU^+$ group, rats that received PNI surgery and LIFU stimulation in parallel, except that the ultrasound (US) amplifier was always turned off during treatment in the $LIFU^-$ group.

2.3. PNI Model of NP. We developed the PNI model using the selective nerve injury (SNI) method in strict accordance with the literature [39]. Rats were anesthetized by intraperitoneal (i.p.) injection of 1% sodium pentobarbital (40 mg/kg). We shaved the fur at the right knee joint's proximal end and made a 1 cm incision. The muscle was separated bluntly, layer by layer, followed by exposure of the three branches of the right sciatic nerve: the tibial nerve, the common peroneal nerve, and the sural nerve. The common peroneal and tibial nerves were loosely ligated with 4-0 silk in three places at 1 mm intervals. We carefully performed manipulations during ligation to avoid injuring the sural nerve. The branches of the right sciatic nerve were exposed but not ligated in sham group rats.

2.4. LIFU Stimulation of the L4–L5 Spinal Cord Section. LIFU stimulation was started on the third day after PNI surgery during the time range of 09 : 00–15 : 00 (Figure 2(a)). After administering mild mixed anesthesia with isoflurane and sodium pentobarbital, we fixed the rats on a table and applied a depilatory cream to remove the fur on their backs, exposing the L4–L5 spinal segment. The transducer was fixed on this segment, and the skin was covered, and the transducer gaps filled with an ultrasonic coupling agent (Aquasonic; Parker Laboratories, Fairfield, NJ, USA) without bubbles. Parameters were as follows: sine pulse wave frequency, 4 MHz; duty

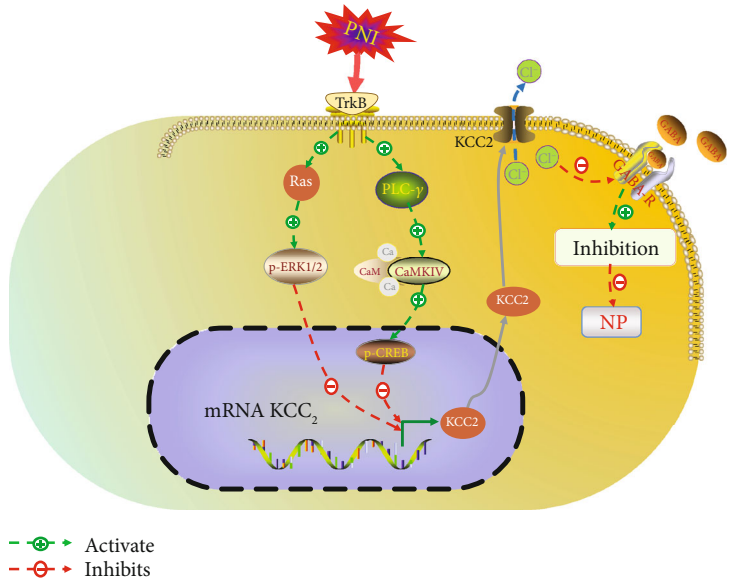


Figure 1: Outline of the current view on the roles of the p-ERK–KCC_2 and CaMKIV–KCC_2 signaling pathways after PNI in the induction of NP. Under normal conditions, KCC_2 extrudes intracellular Cl^- ions from the cell and maintains the inhibitory effect mediated by GABA receptor. PNI activates TrkB and then obstructs the translation of KCC_2 through the p-ERK–KCC_2 and CaMKIV–KCC_2 signaling pathways.

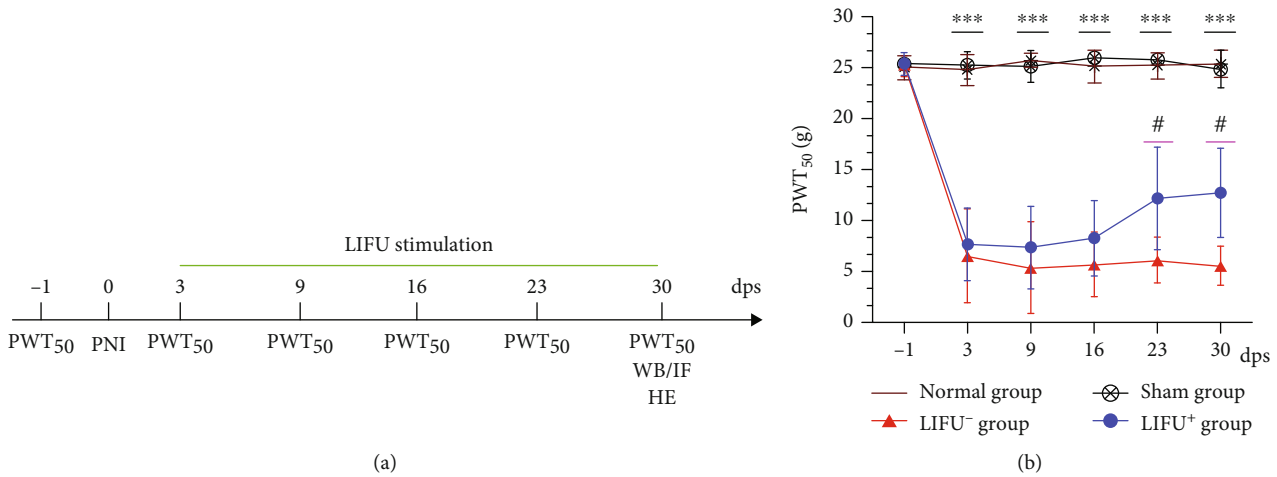


Figure 2: (a) Timeline of experimental protocol. Dps: days postsurgery. (b) Therapeutic effects of LIFU stimulation on NP in PNI rats. Mechanical allodynia (PWT_{50}) was significantly decreased in the $LIFU^-$ and $LIFU^+$ groups 3 days after PNI surgery compared with the normal and sham groups. After 3 weeks of LIFU treatment, PWT_{50} increased compared with the $LIFU^-$ group. Each symbol represents the mean ± SEM; $^{***}P < 0.001$ against the $LIFU^-$ and $LIFU^+$ groups, $\#P < 0.05$ against the $LIFU^-$ group. One-way ANOVA; $n = 10$ per group.

cycle (DC), 20%; pulse repetition frequency (PRF), 0.8 KHz; irradiation intensity, 0.65 MPa; and treatment duration, 20 min/d for 4 weeks. We calibrated the beam's irradiation intensity using a hydrophone (HNR 0500; Onda, Sunnyvale, CA, USA).

2.5. Tissue Preparation. After the last LIFU treatment and behavioral test, rats were sacrificed via overdose of 1% sodium pentobarbital (40 mg/kg), and tissues were harvested for WB ($n = 5$) and IF ($n = 5$) staining analysis. For WB, we rapidly collected L4–L5 spinal cord section tissues and stored them at −80°C until use. For IF, rats were perfused with 200 ml prechilled 0.9% saline (4°C) and then 150 ml prechilled 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde (4°C). We harvested the L4–L5 spinal cord section, fixed it in 4% paraformaldehyde overnight at 4°C, and separately dehydrated the slices one by one for 24 h using 20 and 30% sucrose 0.9% saline solution. After being embedded with optimal cutting temperature (OCT) compound, the transverse section slice (8–12 μm thick) of the spinal cord was used for IF or hematoxylin and eosin (H&E) staining.

2.6. Assessment of LIFU Safety. We performed H&E staining to assess the safety of LIFU for the spinal cord. Sections were prepared according to the following procedures: fixation for 30 s, washing in water for 5 min, staining with hematoxylin

solution for 5 min, dipping in 1% acid ethanol five times, staining with eosin solution for 2 min, dipping in graded alcohol (from a high to a low concentration) for 5 min per grade, washing in water for 15 min, dehydration with graded (from a low to a high concentration) alcohol, clearing with xylene, and mounting in resin. We used a digital microscope to observe the results of H&E staining.

2.7. Measurement of Mechanical Allodynia. Behavioral tests were performed in a controlled environment by investigators who were blinded to animal treatments. Each rat was separately placed in a metallic mesh cage ($20 \times 20 \times 15\ cm^3$) and allowed to adapt to the environment for 20 min before the test. We used the up-and-down method to test PWT_{50} as described in the literature [40]. A series of von Frey (VF) filaments (Stoelting, Wood Dale, IL, USA) with ascending degrees of stiffness (1.4, 2.0, 4, 6, 8, 10, 15, and 26 g) were used to irritate the ipsilateral plantar surface of the PNI paw. The first VF filament to be used was the 6 g filament, and appropriate force was used to bend each filament for 5 s. Licking, lifting, or removing the paw was considered a positive reaction. According to the negative or positive response, we applied a filament at a greater or lower degree of force. PWT_{50} was calculated as follows:

$$50\%\text{g threshold} = \left(10^{[xf+k\delta]}\right)/10,000. \quad (1)$$

The PWT_{50} test was performed presurgery for 1 day, and pre-LIFU stimulation was performed 1 day/week during the LIFU stimulation period.

2.8. Western Blotting (WB) Analysis. The spinal cord tissue (0.1 g) was dissected, homogenized via US, lysed with radioimmunoprecipitation assay (RIPA) buffer (RIPA: phenylmethylsulfonyl fluoride [PMSF] = 1 ml : 10 μl) on ice for 30 min, and centrifuged at 12,000 r/min for 30 min at 4°C; then, we harvested the supernatants. Total protein concentration was quantified via a bicinchoninic acid (BCA) assay kit (Biomed, Beijing, China), and all samples were equalized to 30 μg/10 μl. Samples (total protein, 30 μg) were resolved by 6%, 10%, and 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore-Sigma, Burlington, MA, USA). We blocked the membranes with 5% fat-free milk at room temperature (RT) for 2 h and then incubated them overnight with primary antibodies at 4°C with gentle shaking. These antibodies included monoclonal antibodies (mAbs) against CaMKIV (1: 2000; Abcam, Cambridge, UK), p-CREB (1: 1000; Cell Signaling Technology [CST], Danvers, MA, USA), p-ERK1/2 (1: 2000; CST), and KCC_2 (1: 1000, CST), as well as glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1: 50,000; ABclonal Technology, Woburn, MA, USA) and β-actin (1: 2000; Santa Cruz Biotechnology, Dallas, TX, USA). The membranes were then incubated with a secondary antibody, horseradish peroxidase- (HRP-) labeled anti-rabbit/anti-mouse immunoglobulin G (IgG) HRP-linked antibody (1: 2000; CST), for 90 min at RT. Finally, we visualized and quantified protein bands using enhanced chemiluminescence (ECL; Tanon, Shanghai, China) and an ImageJ software (US National Institutes of Health [NIH], Bethesda, MD, USA). Protein was normalized based on β-actin or GAPDH concentrations.

2.9. Immunofluorescence (IF) Staining. For IF, each slice was washed in phosphate-buffered saline (PBS) for 10 min at RT and then incubated with 5% goat serum and 0.03% Triton X-100 in 0.1 M PBS for 2 h. Then, we incubated the slices in primary antibodies against KCC_2 and p-CREB (respectively, 1 : 100 and 1 : 800; CST), as well as antibody against NeuN (1 : 1000, Abcam), at 4°C overnight. The secondary antibodies (anti-rabbit IgG [heavy + light (H + L) chain], F [ab′]2 fragment [Alexa Fluor 488 Conjugate]; anti-mouse IgG [H + L chain], F [ab′]2 fragment [Alexa Fluor 594 Conjugate]) were used for incubation at RT in the dark for 2 h. After three 10 min washes with PBS, we incubated the sections with 4′,6-diamidino-2-phenylindole (DAPI; Solarbio, Beijing, China). Images were captured under a fluorescence microscope (Olympus Corp., Tokyo, Japan). We used ImageJ software (US National Institutes of Health [NIH], Bethesda, MD, USA) to quantify the density of positive regions.

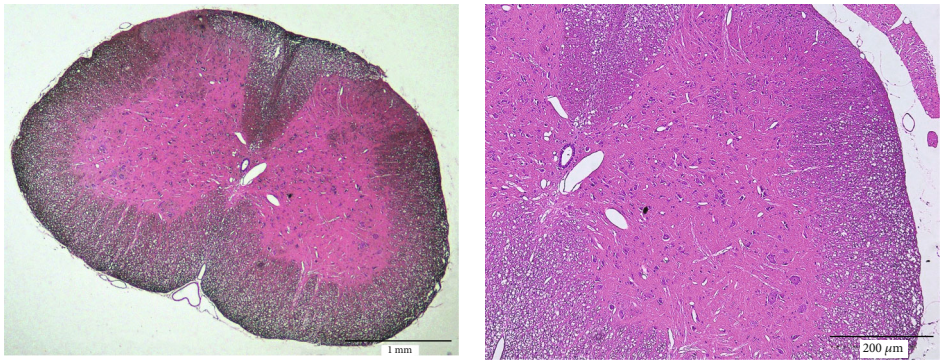
2.10. Statistical Analyses. Data are presented as mean ± standard deviation (SD). We used SPSS version 23.0 (IBM Corp., Armonk, NY, USA) for all statistical analyses. GraphPad Prism software version 8.0 (GraphPad Software, Inc., San Diego, CA, USA) was used to generate graphs. After verifying that all data were normally distributed, we used one-way analysis of variance (ANOVA) to analyze PWT_{50}, WB, and IF data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. H&E Staining of the L4–L5 Spinal Cord Section Was Used to Observe the Safety of LIFU Stimulation. We saw no swelling or nuclear fragmentation of neurons, neutrophil infiltration, or bleeding under cross-sectional magnification (Figure 3(a), ×40; Figure 3(b), ×100) of this spinal cord section.

3.2. LIFU Alleviated Mechanical Allodynia in PNI Model Rats. As shown in Figure 2(a), we used PWT_{50} to assess the effect of LIFU stimulation on PNI rats at different times. One day before LIFU stimulation, PWT_{50} had significantly decreased from 25.3 ± 1.2 g ($LIFU^-$ group) and 25.4 ± 1.1 g ($LIFU^+$ group) to 6.6 ± 4.6 g and 7.7 ± 3.6 g, respectively ($P < 0.05$), but there was no statistically significant difference between the two groups ($P > 0.05$). After LIFU stimulation, PWT_{50} gradually increased, eventually becoming higher in the $LIFU^+$ group (12.1 ± 5.0 g) than in the $LIFU^-$ group (6.1 ± 2.2 g) after 3 weeks of LIFU stimulation ($P < 0.05$) and remaining stable to the end of LIFU stimulation. However, it was still lower in the normal and sham operation groups ($P < 0.05$), which there was no significant difference ($P > 0.05$; Figure 2(b)).

3.3. LIFU Stimulation Increased the KCC_2 Expression in the L4–L5 Spinal Cord Section. After 4 weeks of LIFU stimulation, rats were sacrificed, and the L4–L5 spinal cord section was harvested for WB (Figure 4(a)) and IF (Figure 5(a))



(a) (b)

Figure 3: H&E staining showed that LIFU was safe for stimulating the spinal cord ((a) ×40, scale bar = 1 mm; (b) ×100, scale bar = 200 μm) L4–L5 section of the spinal cord, showing no edema, hemorrhage, or cell necrosis.

analyses. The results showed that the expression of the KCC_2 protein in rats in the $LIFU^+$ group was upregulated compared with those in the $LIFU^-$ group ($P < 0.05$). There was no difference between the normal and sham groups ($P > 0.05$; Figures 4(b) and 5(b)).

3.4. LIFU Stimulation Reduced the Expression of CaMKIV and p-CREB but Not of p-ERK1/2 in the L4–L5 Spinal Cord Section of PNI Rats. PNI activates the MAPK pathway and leads to high expression of CaMKIV, p-ERK, and p-CREB [41]. In this study, WB (Figures 4(c)–4(h)) showed that the expression of CaMKIV, p-ERK1/2, and p-CREB increased in the $LIFU^-$ group. After 4 consecutive weeks of LIFU treatment, the expression of CaMKIV and p-CREB decreased compared with the $LIFU^-$ group ($P < 0.05$; Figures 4(f) and 4(h)). IF also showed that the expression of p-CREB decreased after LIFU stimulation for 4 weeks compared with the $LIFU^-$ group ($P < 0.05$; Figure 5(d)). Interestingly, there was no statistical difference in the p-ERK1/2 expression between the $LIFU^-$ and $LIFU^+$ groups, nor any significant difference in CaMKIV, p-ERK1/2, or p-CREB expression between the normal and sham groups ($P > 0.05$; Figures 4(d), 4(f), and 4(h)).

4. Discussion

Potassium chloride (K^+-Cl^-) cotransporter 2 (KCC_2) is the only cationic chloride cotransporter expressed in mammalian neurons. It plays a prominent role in maintaining low $[Cl^-]_i$, which is necessary for the function of $GABA_A$ and glycine receptors (GlyRs) and for mediating spinal cord inhibition [8, 10]. After intrathecal application of KCC_2 inhibitor (2-[[(2S)-2-butyl-6,7-dichloro-2-cyclopentyl-1-oxo-3H-inden-5-yl]oxy], or DIOA), heat-evoked withdrawal latency and innocuous brush stimulation are significantly reduced [16, 42]. Our experimental data indicated that KCC_2 was downregulated in the PNI group, and PWT_{50} was also lower in this group than in the normal and sham operation groups. All results showed that PNI led to downregulation of the KCC_2 expression, which weakens $GABA_A$/GlyR-mediated inhibition and then leads to NP [43]. All of the above changes are important factors contributing to the development and maintenance of NP. To further investigate the mechanism of NP, we found that enhancing the KCC_2 function pharmacologically restored spinal cord inhibition and reduced allodynia [9]. In our study, pain behavior improved (Figure 2(b)), and the KCC_2 expression was upregulated (Figure 4(b)) after 4 weeks of LIFU stimulation. Therefore, the expression of KCC_2 in the spinal cord played an important role in the pathogenesis of NP, and upregulation of the KCC_2 expression could potentially alleviate NP.

After PNI, the downregulation of KCC_2 is closely related to activation of the BDNF–TrkB pathway and intracellular cascade reactions mediated by CaMKIV, p-CREB, and p-ERK [21–24]. Intrathecal application of a TrkB blocker significantly improves downregulation of the KCC_2 expression on the membranes of spinal dorsal horn neurons induced by inflammatory pain [44]. In Kitayama's research, short interfering RNA (siRNA) was used to knock down zinc transporter-1 (ZnT-1), which led to inhibition of the BDNF–TrkB pathway, downregulation of p-CREB, upregulation of KCC_2, and improvement of the withdrawal threshold [19]. After intrathecal injection of p-ERK blockers, chronic NP induced by oxaliplatin was also significantly alleviated in rats [45]. López-Alvarez and Li applied electroacupuncture to stimulate rats with chronic constriction injury (CCI) and found that it could effectively improve the KCC_2 expression, the mechanical withdrawal threshold, and thermal withdrawal latency [18, 20]. Therefore, inhibition of the BDNF–TrkB pathway and cascade reactions mediated by CaMKIV, p-CREB, and p-ERK, as well as upregulation of the KCC_2 expression, could effectively alleviate NP. In this study, we stimulated the spinal cord with LIFU and confirmed the efficacy of LIFU in treating NP. To our knowledge, this study was the first to use LIFU to stimulate the spinal cord in order to regulate NP.

Moreover, we found that CaMKIV and p-CREB were downregulated (Figures 4(f), 4(h), and 5(d)), and KCC_2 upregulated (Figures 4(a) and 5(b)) after LIFU stimulation. Upregulation of the KCC_2 expression can reduce neural $[Cl^-]_i$, increase the effect of GABA, and enhance the inhibitory effect of interneuron on the spinal cord, so that the pain threshold of sensory neurons in the spinal cord is reduced

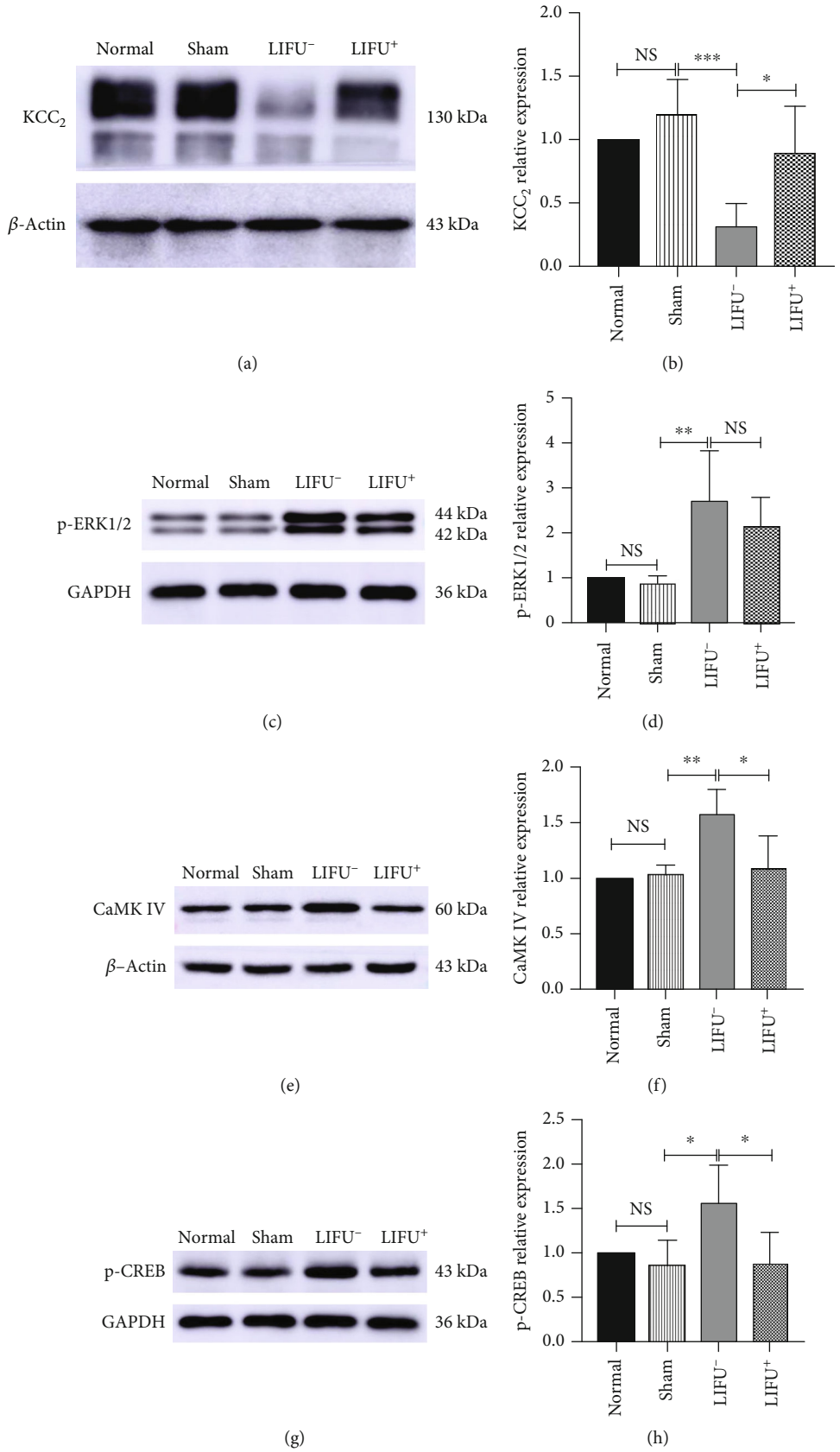


FIGURE 4: WB analysis of KCC_2 (a, b), p-ERK1/2 (c, d), CaMKIV (e, f), and p-CREB (g, h) expression in the L4–L5 section of the spinal cord in different groups at 4 weeks post-LIFU treatment. Values, normalized to β-actin, or GAPDH. Each symbol represents the mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. One-way ANOVA; $n = 5$ rats per assay.

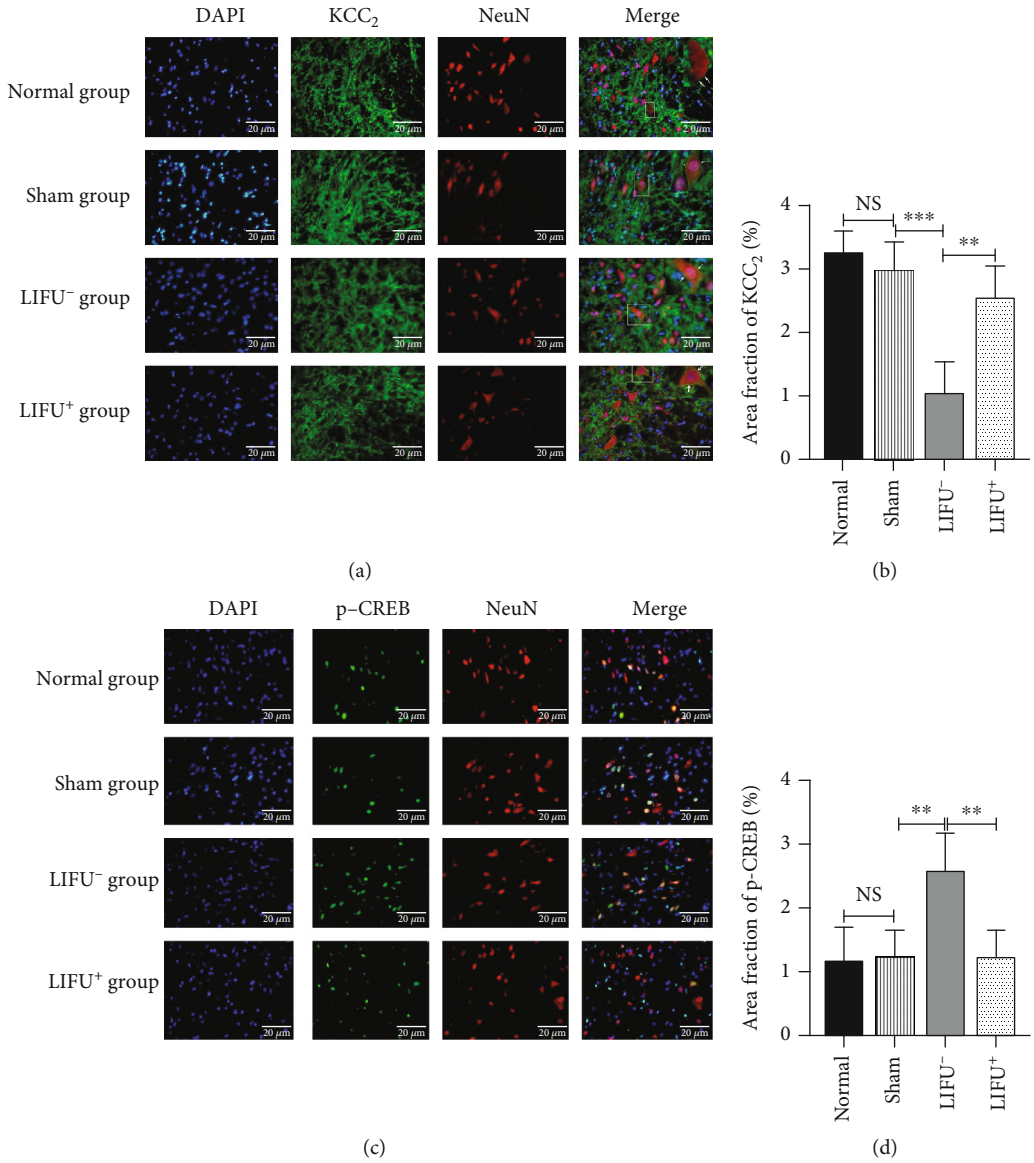


Figure 5: Expression of KCC_2 (a) p-CREB (c) in the spinal cords of rats in different groups (IF, ×400). Scale bar = 20 μm. Intensities of KCC_2 (b) and p-CREB (d) IF in the spinal cords of rats in different groups after 4 weeks of LIFU treatment. Each symbol represents the mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. One-way ANOVA; $n = 5$ rats per assay.

and the behavior of pathological pain is relieved [7, 46]. Therefore, we speculate that LIFU might alleviate pathological pain due to PNI by inhibiting CaMKIV and p-CREB expression and upregulating the KCC_2 expression in neurons.

Interestingly, LIFU stimulation did not change the expression of p-ERK1/2 in the spinal cords of PNI rats (Figure 4(d)). While the exact underlying mechanism is unknown, there are several possible explanations. First, in NP rat models, the BDNF–TrkB pathway can activate CaMKIV by increasing the concentration of Ca^{2+} in neurons via the PLCγ–IP_3 pathway, whereas p-ERK is activated through the TrkB–Ras pathway [22, 26]. Second, CaMKIV activation depends on the concentration of Ca^{2+} in neurons. The mechanical forces of LIFU can affect voltage-gated calcium and sodium channels (VGCCs, VGSCs) in the plasma membrane [35, 47], causing transient intracellular Ca^{2+} concentration changes in various cells [48]. This mechanism can be used in treatments such as mesenchymal stem cell (MSC) homing [36], neuromodulation in the brain [47], or immunotherapy with tumor US [49]. Therefore, we propose that LIFU might affect CaMKIV activation by interfering with the transient concentration of Ca^{2+} in neurons but without affecting the p-ERK1/2 expression. However, the specific mechanism of action remains unclear, requiring further research.

As a noninvasive neuromodulatory method, LIFU has many advantages such as higher spatial resolution, greater penetration depth, and no tissue damage [50]. As a nonthermal form of US, LIFU has litter thermal effect on local tissues. When peripheral focused US (pFUS; $F = 1.15$ MHz; peak negative pressure [PNP] = 4 MPa; DC = 5%) that is used to irradiate muscle and kidney tissue in vitro, the temperatures of these tissues increase by 1.1°C and 0.7°C, respectively [36].

In this study, we transected the spinal cord at L4–L5 and performed H&E staining to observe the safety of LIFU on the spinal cord. Our results showed no swelling, nuclear fragmentation of neurons, neutrophil infiltration, or bleeding (Figures 3(a) and 3(b)). Therefore, these results indicated that LIFU was a safe method for treating the spinal cord.

Overall, our study demonstrated that (i) LIFU stimulation of the spinal cord could effectively improve neuropathic pain behavior induced by peripheral nerve injury, which has potential value in the clinical treatment of NP; (ii) LIFU stimulation of the spinal cord might affect the expression of CaMKIV, CREB, and KCC_2; and (iii) stimulation of the spinal cord with LIFU was safe.

5. Limitations

Our study had some limitations. First, we established only a short treatment period and did not evaluate the long-term efficacy of US therapy. Second, we selected only one time point at which to measure the expression of CaMKIV, p-CREB, p-ERK, and KCC_2. Third, we found that LIFU could affect the expression of CaMKIV, p-CREB, and KCC_2, but we failed to explore the specific mechanism by which it affected the expression of the above proteins. Thus, further experiments are needed.

6. Conclusions

We found that LIFU could effectively alleviate NP in rats with PNI by increasing the expression of KCC_2 in the spinal dorsal corner. Moreover, LIFU upregulated the expression of KCC_2, possibly by inhibiting activation of the CaMKIV–KCC_2 pathway.

Abbreviations

NP: Neuropathic pain
LIFU: Low-intensity focused ultrasound
PNI: Peripheral nerve injury
KCC_2: Potassium chloride cotransporter 2
CaMKIV: Calcium/calmodulin-dependent protein kinase type IV
p-CREB: Phosphorylated cyclic adenosine monophosphate response element-binding protein
p-ERK1/2: Phosphorylated extracellular signal–regulated kinase 1/2
GABA: γ-Aminobutyric acid
BDNF: Brain-derived neurotrophic factor
TrkB: Tropomyosin receptor kinase B
CNS: Central nervous system
dps: Days postsurgery
PWT_{50}: 50% paw withdrawal threshold.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

The Prediction of Acute Postoperative Pain Based on Neural Oscillations Measured before the Surgery

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Even with an improved understanding of pain mechanisms and advances in perioperative pain management, inadequately controlled postoperative pain remains. Predicting acute postoperative pain based on presurgery physiological measures could provide valuable insights into individualized, effective analgesic strategies, thus helping improve the analgesic efficacy. Considering the strong correlation between pain perception and neural oscillations, we hypothesize that acute postoperative pain could be predicted by neural oscillations measured shortly before the surgery. Here, we explored the relationship between neural oscillations 2 hours before the thoracoscopic surgery and the subjective intensity of acute postoperative pain. The spectral power density of resting-state beta and gamma band oscillations at the frontocentral region was significantly different between patients with different levels of acute postoperative pain (i.e., low pain vs. moderate/high pain). A positive correlation was also observed between the spectral power density of resting-state beta and gamma band oscillations and subjective reports of postoperative pain. Then, we predicted the level of acute postoperative pain based on features of neural oscillations using machine learning techniques, which achieved a prediction accuracy of 92.54% and a correlation coefficient between the real pain intensities and the predicted pain intensities of 0.84. Altogether, the prediction of acute postoperative pain based on neural oscillations measured before the surgery is feasible and could meet the clinical needs in the future for better control of postoperative pain and other unwanted negative effects. The study was registered on the Clinical Trial Registry (https://clinicaltrials.gov/ct2/show/NCT03761576?term=NCT03761576&draw=2&rank=1) with the registration number NCT03761576.

1. Introduction

More than 230 million major surgeries are performed annually around the world [1]. Even with an improved understanding of pain mechanisms and advances in perioperative pain management, inadequately controlled postoperative pain continues. In fact, the incidence of moderate-to-severe acute postoperative pain ranges from 20% to 80% [2]. Postoperative pain would lead to a series of negative outcomes, including delayed recovery time, increased cost of care, and an increased incidence of the transition from acute pain to chronic pain [1]. One way to prevent these issues is to adopt more effective analgesic strategies in the perioperative period. More effective analgesia could be achieved with the help of successful predictions of acute postoperative pain based on physiological measures before the surgery. Exploiting the power of machine learning techniques, we could identify patients at risk by predicting acute postoperative pain based on physiological measures before the surgery. The correct prediction would help deepen our understanding of the biological underpinning of the risk. In addition, the prediction would help develop more targeted or preventative treatments (i.e., individualized treatments) to improve analgesic efficacy [3, 4].

Pain is a sensory and emotional experience with a high level of variability across different individuals [5]. Importantly, pain perception is closely related to neural oscillations [6], which play a crucial role in the segregation and

integration of brain regions for functioning [7]. Specifically, numerous studies of acute and chronic pain using electroencephalography (EEG) and magnetoencephalography (MEG) highlighted the important role of neural oscillations at theta, alpha, beta, and gamma frequency bands in characterizing pain perception [6, 8–13], although the specificity of the relationship between neural oscillations and pain is disputed. The relationship between pain perception and neural oscillations could be summarized in three main aspects. First, nociceptive stimuli can induce significant modulations of neural oscillations at theta, alpha, beta, and gamma frequency bands [5, 6, 13, 14], and importantly, the changes of the magnitude of some neural oscillations are robustly correlated with the subjective intensity of pain perception [5, 13, 15]. For example, gamma oscillations recorded from the primary somatosensory cortex could predict the subjective pain intensity within the same individual and encode pain sensitivity across different individuals [5]. In addition, gamma oscillations in the prefrontal cortex are likely to encode subjective pain perception of tonic heat pain [10, 16]. Second, neural oscillations before nociceptive stimuli could also predict the subjective intensity of pain perception that is evoked by the forthcoming nociceptive stimuli [17, 18]. Specifically, alpha oscillations at bilateral central regions and gamma oscillations at parietal regions act synergistically and causally in predicting the intensity of pain perception elicited by subsequent nociceptive stimuli [17]. Third, altered neural oscillations are observed in many chronic pain conditions, such as fibromyalgia [8, 13], chronic back pain [9], and postherpetic neuralgia [12]. For example, one MEG study found that fibromyalgia patients exhibited increased beta and gamma power in the dorsolateral prefrontal and orbitofrontal cortex [8]. Interestingly, increased power in these two frequency bands also correlated with higher affective pain scores in fibromyalgia patients [8].

Considering the strong correlation between pain perception and neural oscillations, we hypothesize that acute postoperative pain could be predicted by neural oscillations measured shortly before the surgery. In practice, this research aim could be achieved based on the combination of neuroimaging techniques to record neural oscillations and machine learning techniques [19, 20] to predict postoperative pain. Machine learning is referred to as a set of algorithms that can automatically detect patterns from neuroimaging data and utilize the detected patterns to predict clinical outcomes [21, 22], e.g., the intensity of acute postoperative pain. Accumulating evidence has been documented to show that machine learning is able to extract meaningful information from high-dimensional and noisy neuroimaging data, thus effectively identifying neural markers for behaviors and diseases [21, 22].

Here, neural oscillations were measured using the EEG technique shortly before the surgery, for which the technique is clinically feasible in most situations as EEG can be used directly at the patients' bedside. The relationship between neural oscillations before the surgery and the subjective intensity of acute postoperative pain was quantified using spectral analysis and partial correlation analysis. Afterward, machine learning techniques were applied to predict the intensity of postoperative pain based on EEG recordings shortly before the surgery. Ideally, surgery patients with a high risk of postoperative pain could be identified before the surgery, which could provide a vital measure to optimize the analgesic strategy for better control of the postoperative pain and other negative outcomes.

2. Methods

The clinical study was conducted at the Department of Anesthesiology of the Peking University People's Hospital, Beijing, China. The Medical Ethics Committee of the Peking University People's Hospital approved the study protocol. A written informed consent was obtained from all participants. The study was registered on the Clinical Trial Registry (https://register.clinicaltrials.gov/) with the registration number NCT03761576.

2.1. Participants. Patients admitted to the Peking University People's Hospital for lobectomy, wedge resection, or mediastinotomy under thoracoscopic surgery were recruited between November 2018 and March 2019. Inclusion criteria were (1) age between 35 and 65 years, (2) education levels beyond secondary school, (3) American Society of Anesthesiologist (ASA) grade I-II, (4) preferred to use postoperative patient-controlled analgesia (PCA), and (5) signed informed consent. Exclusion criteria were (1) neurological diseases, (2) psychiatric diseases or psychiatric family history, (3) traumatic brain injury or postcraniotomy, (4) chronic pain suffers or preoperative opioid users, and (5) thoracotomy needed or planned to return to Intensive Care Unit after surgery. The detailed demographic information is summarized in Table 1.

2.2. Study Design. As showed in Figure 1(a), the study design is composed of three phases. In phase 1, patients were instructed to sign the informed consent one day before their surgery, and patients were required to avoid smoking or drinking coffee or caffeine-containing beverages 10 hours before the surgery. In phase 2, resting-state EEG data were collected from all patients 2 hours before the surgery (please see the following section for details about EEG data collection), and all patients were instructed to complete the Hospital Anxiety and Depression Scale (HADS) before EEG data collection. In phase 3, postoperative pain on the 1st, 2nd, and 3rd days after the surgery was collected from all patients. Specifically, the highest postoperative pain over the past 24 hours was assessed on an 11-point numerical rating scale (NRS) (0 = no pain, 10 = worst pain imaginable) at 10-14 o'clock on the 1st and 2nd days after the surgery. On the 3rd day after the surgery, the highest postoperative pain over the past 24 hours was assessed using the same NRS, but after the chest analgesic tube was removed. Please note that the highest postoperative pain over the past 24 hours was obtained by the evaluation of pain at rest and pain due to movements, e.g., coughing and breathing. As recommended by Zalon in 2014 [23], clinicians should actively intervene with patients with a pain score (i.e., NRS scores) more than 3. For this reason, patients with NRS scores higher than 3

Table 1: Participant demographic information and postoperative pain.

Variables	Categories	Moderate/high-pain group ($n = 33$)	Low-pain group ($n = 34$)	p value
Gender	Male	14	15	0.729
	Female	20	18	
Age (year)		55.09 ± 6.14	56.45 ± 7.67	0.086
Education level	Junior	6	7	0.277
	High school	13	5	
	College	15	21	
ASA grade	I	13	17	0.281
	II	21	16	
Operation type	Thoracoscopic wedge resection	15	15	0.658
	Thoracoscopic lobectomy	15	16	
	Thoracoscopic mediastinotomy	4	2	
HADS score	Anxiety score	5.06 ± 3.06	4.3 ± 3.02	0.458
	Depression score	5.61 ± 3.79	5.65 ± 3.65	0.506
Dose of oxycodone (mg)		14.03 ± 12.03	8.09 ± 7.44	0.021*
NRS on the 1st day		5.21 ± 1.76	4.41 ± 1.81	0.072
NRS on the 2nd day		5.33 ± 1.93	3.53 ± 1.99	<0.001**
NRS on the 3rd day		5.18 ± 1.76	2.24 ± 0.86	/

*$p < 0.05$; **$p < 0.001$.

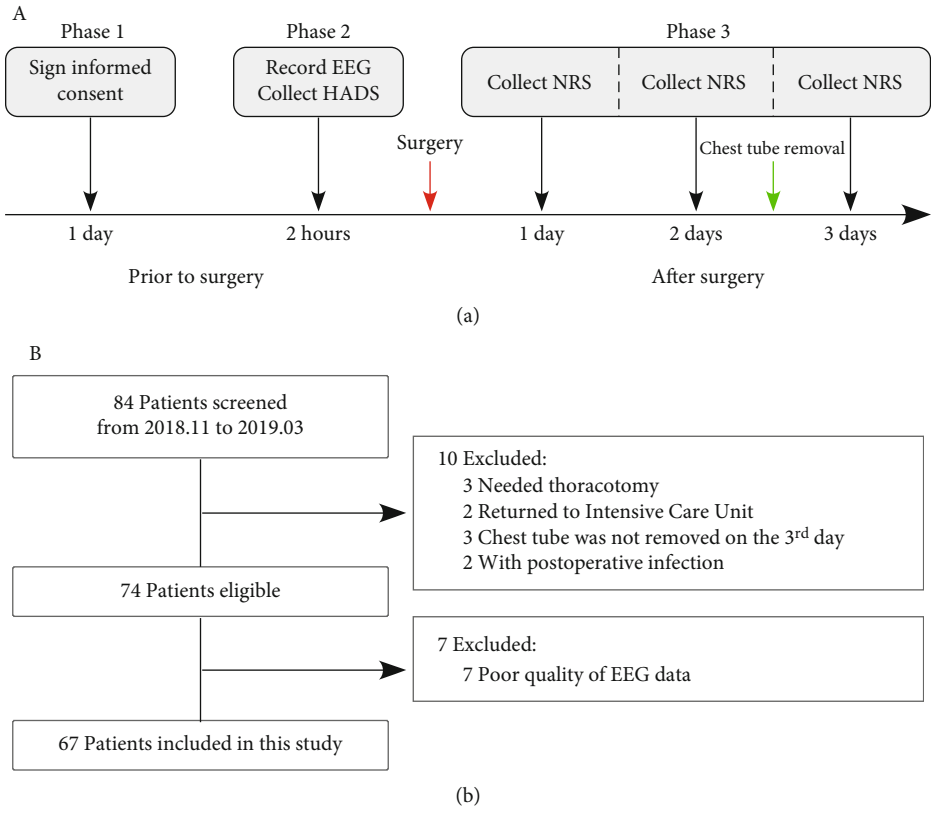

Figure 1: (a) Study design and the (b) flow of participants.

on the 3rd day after the surgery were considered with moderate/high pain, while patients with NRS scores of 3 or lower than 3 were considered without low pain. All patients were examined by the same investigator, and all patients were reminded that they could withdraw from the experiment at any time for any reason, but none did so.

Please note that after EEG data collection, patients first received local anesthesia (i.e., thoracic paravertebral block at T4 and T7 on the affected side) and then received general anesthesia according to the local clinical standards to ensure the safety of the surgery. Patients received thoracic paravertebral block at T4 and T7 on the affected side with an infusion of 0.4% bupivacaine (20 ml) before general anesthesia. The induction of anesthesia was achieved using midazolam (0.02-0.04 mg/kg), propofol (1-2 mg/kg), and sufentanil (0.2-0.4 μg/kg). Then, rocuronium (0.6-1 mg/kg) was intubated with a double-lumen endotracheal tube, for which the position was confirmed by fiberoptic bronchoscopy. Anesthesia was maintained with 1% sevoflurane, propofol (0.1-0.3 μg/kg/min), and remifentanil (0.1–0.3 μg/kg/min) during the surgery. More sufentanil and rocuronium were supplied when needed, and the total amount of sufentanil should be no more than 0.6 μg/kg. Flurbiprofen axetil (100 mg) was started to be administrated 30 min before the end of the surgery. Along with the infusion of flurbiprofen axetil (8 mg/h), the patient-controlled analgesia (PCA) with oxycodone (Perfusor fm PCA; single dose 1 mg, lockout 5 min, limit 8 mg/h) was used for all patients as soon as they were able to operate the system.

2.2.1. EEG Recording. Patients lay in a bed with a semirecumbent position in a silent, temperature-controlled room. The EEG cap was mounted on their head with conducting gel inserted for each electrode, and all electrode impedances were kept lower than 10 kΩ. EEG data were recorded using a 32-channel NuAmps Quickcap, NuAmps DC amplifier, and Scan 4.5 Acquisition software (Compumedics Neuroscan, Inc. Charlotte, NC, USA). The NuAmps amplifier (Model 7181) was set with a sampling rate of 1000 Hz and with a signal bandpass filter from 0.01 to 100 Hz. The ground electrode was positioned 10 mm anterior to Fz, and the right mastoid electrode (M2) was used as the online reference. During EEG data collection (five minutes in total), all subjects were instructed to keep awake, relaxed, and eyes closed, since the test-retest reliability of resting-state EEG data was higher in the eyes closed condition than in the eyes open condition [24].

2.2.2. EEG Preprocessing. EEG data were preprocessed using EEGLAB [25]. Continuous EEG data were first offline rereferenced to the average bilateral mastoid electrodes (M1 and M2). Then, EEG data were bandpass filtered between 0.5 and 80 Hz and notch filtered between 48 and 52 Hz. For the artifact rejection, continuous EEG data were segmented into epochs using a time window of 5 s. EEG epochs were decomposed into a series of independent components (ICs) using the infomax algorithm as implemented in EEGLAB [25]. The number of ICs was equal to the number of EEG electrodes. ICs contaminated by eye blinks and movements were identified and removed using the SASICA algorithm [26, 27]. The number of the removed ICs was comparable for the low-pain and moderate/high-pain groups (2.7 ± 0.22 and 2.3 ± 0.21, respectively, $p = 0.19$). Moreover, epochs contaminated by gross artifacts (i.e., exceeding $\pm 75\,\mu$V in any channel) were automatically rejected. The proportion of epochs rejected was not significantly different between the low-pain and moderate/high-pain groups ($19 \pm 4.5\%$ and $22 \pm 3.9\%$, respectively, $p = 0.6$).

2.2.3. EEG Spectral Analysis. For each patient, the preprocessed EEG data were transformed to the frequency domain using Welch's method (window length: 2 s; overlap: 50%) [28], yielding an EEG spectrum ranging from 0.5 to 80 Hz, in steps of 0.5 Hz. Group-level EEG spectra were obtained by calculating the average of single-patient EEG spectra in each group (i.e., moderate/high-pain group and low-pain group). To assess the group difference of EEG spectra, a point-by-point independent-sample t-test was performed for each frequency (across all frequency bins) and each electrode, and the significant level (p value) was corrected using a false discovery rate (FDR) procedure [29]. Additionally, to control for false-positive observations, the frequency intervals with a p value smaller than the defined threshold ($p_{\text{fdr}} < 0.05$) for more than 5 Hz were considered as significant. Partial correlation analysis was also performed between EEG power at different frequency bands and acute postoperative pain (i.e., NRS scores on the 3rd day after the surgery) to assess their relationship while controlling for the effect of age and removing the possible outliers. Please note that the outliers were identified using the threshold of three standard deviations of EEG power, i.e., the data was identified as an outlier if its value was three standard deviations away from the mean [30].

2.2.4. Machine Learning: Classification and Regression. We performed the linear discriminant analysis (LDA) [31], a typical machine learning algorithm, to predict the intensity of postoperative pain based on EEG recordings shortly before the surgery. Considering the arbitrary nature of dichotomizing the two groups, we also predicted the continuous pain ratings (i.e., the intensity of postoperative pain) using the multiple linear regression (MLR) [32]. Leave-One-Out Cross-Validation (LOOCV) [33] was used to assess the prediction performance. Specifically, LOOCV was achieved by dividing all subjects (N subjects) into $N - 1$ training subjects and 1 test subject, and the same procedure was repeatedly performed N times to ensure that every subject was used as the test subject once. The classification accuracy and correlation coefficient (R) between the real pain intensities and the predicted pain intensities were used to evaluate the prediction performance of LDA and MLR, respectively.

To assess the contribution of EEG feature at each electrode and each frequency on the prediction performance, the LDA and MLR were firstly performed for each electrode in the spatial domain and each frequency in the frequency domain. For both classification and regression, all EEG features were tested once, and the maximal values of prediction accuracy for classification and correlation coefficient for

regression at the electrode level and the frequency level were, respectively, used to evaluate the contribution of these features in the machine learning model.

To achieve better prediction performance, EEG features at all electrodes and all frequencies (i.e., the combination of features at the spatial and frequency domains) were used in the multivariate machine learning model. In the present study, there were 160 features for each electrode (from 0.5 Hz to 80 Hz with a resolution of 0.5 Hz) and 30 electrodes. In total, the feature dimension was 4800 (160 frequency bins × 30 electrodes), and the sample size was 67 (67 patients). This is a typical small sample size pattern recognition problem with a high feature dimension. In this case, the curse of feature dimensionality is the main problem for both classification and regression. To address this issue, feature selection is required before performing prediction. In addition, feature selection is an effective strategy for dimension reduction to prevent overfitting. Here, we firstly shrunk the features in the frequency domain to the range of 20-70 Hz, since there was no significant difference between the two groups for all channels outside the frequency range (i.e., 0.5-20 Hz and 70-80 Hz). Secondly, Sequential Floating Forward Selection (SFFS) method was used as a wrapper approach for additional feature selection [29]. As a heuristic search method [34], the SFFS algorithm starts with an empty feature set and mainly consists of a forward step for inserting features and a backward step for deleting features. The forward step searches the best features outside the feature set to improve the prediction performance in the cross-validation. After each forward step, the backward step removes the feature in the feature set as long as the performance could be improved in the cross-validation. The whole process of the SFFS would stop if the prediction performance could not be improved or the feature dimension reaches 50.

2.2.5. Statistical Analysis. Demographic information of patients in the moderate/high-pain group and the low-pain group was compared using chi-square tests (i.e., gender, education level, ASA grade, and operation type) and an independent-sample t-test (i.e., age). Group differences in HADS scores (i.e., anxiety score and depression score), doses of oxycodone, and postoperative pain (i.e., NRS scores on the 1st, 2nd, and 3rd days after the surgery) were evaluated using independent-sample t-tests. All statistical analyses were carried out in SPSS 25.0 (SPSS Inc., New York, USA), and the statistical significance level was set at 0.05.

3. Results

We screened 84 patients undergoing thoracoscopic surgery for eligibility. As summarized in Figure 1(b), 10 patients were excluded from the study for the following reasons: 3 patients needed thoracotomy, 2 patients returned to the Intensive Care Unit after the surgery, 3 patients for whom the chest analgesic tube was not removed on the 3rd day after the surgery, and 2 patients with postoperative infection (i.e., the body temperature was higher than 38.0°C for more than 2 days). Moreover, data from 7 patients were excluded from the following analysis due to the poor quality of EEG data. As a result, 67 patients were eligible for inclusion. According to the postoperative pain on the 3rd day after the surgery, the eligible patients were assigned to the two groups: moderate/high-pain group ($n = 33$) and low-pain group ($n = 34$). As demonstrated in Table 1, no significant differences were observed between patients in the moderate/-high-pain group and those in the low-pain group for clinical and demographic characteristics, i.e., gender, age, education level, ASA grade, and operation type. In addition, both anxiety and depression scores, as evaluated using the HADS, were not significantly different between patients in the two groups. However, the dose of oxycodone and postoperative pain (e.g., NRS on the 2nd day after the surgery) were significantly higher for patients in the moderate/high-pain group than those in the low-pain group ($p = 0.021$ and $p < 0.001$, respectively).

Group-level spectra of resting-state EEG oscillations in both moderate/high-pain and low-pain groups are showed in Figure 2. The point-by-point statistical analysis revealed that patients in the moderate/high-pain group had significantly higher spectral power density of resting-state EEG oscillations at the frontocentral region (maximal at FCz electrode) within beta and gamma frequency bands (between 21 and 55 Hz) than patients in the low-pain group ($p_{\text{fdr}} < 0.05$). When taken separately, beta (14-30 Hz) and gamma (31-50 Hz) band powers in the moderate/high-pain group were both significantly higher than those in the low-pain group (see Figure 3; beta band: $t = 2.063$, $p = 0.043$; gamma band: $t = 2.935$, $p = 0.005$). To test the robustness of results, we also performed partial correlation analysis between EEG power at beta and gamma frequency bands and acute postoperative pain (i.e., NRS scores on the 3rd day after the surgery) while controlling for the effect of age and removing the possible outliers which lay three standard deviations away from the mean. Both beta band power (partial $R = 0.25$, $p = 0.04$) and gamma band power (partial $R = 0.29$, $p = 0.02$) were significantly correlated with acute postoperative pain (Figure 3).

The contribution of presurgery EEG features on the performance of machine learning algorithms to predict the intensity of postoperative pain is displayed in Figure 4. EEG features in the frequency domain showed distinct patterns of contribution to the prediction performance for classification (i.e., LDA, Figure 4(a)) and regression (i.e., MLR, Figure 4(b)). However, the features around 30 Hz provided the most discriminative information for both classification and regression. As displayed in Figures 4(c) and 4(d), EEG features in the frontocentral region were more discriminative for both classification and regression. For classification, electrodes Cz, FCz, and F3 provided the highest prediction accuracy. For regression, electrodes F3, F4, FCz, and Fz provided the highest correlation coefficient.

To achieve better prediction performance, EEG features at all electrodes and all frequencies (i.e., the combination of features at the spatial and frequency domains) were used in

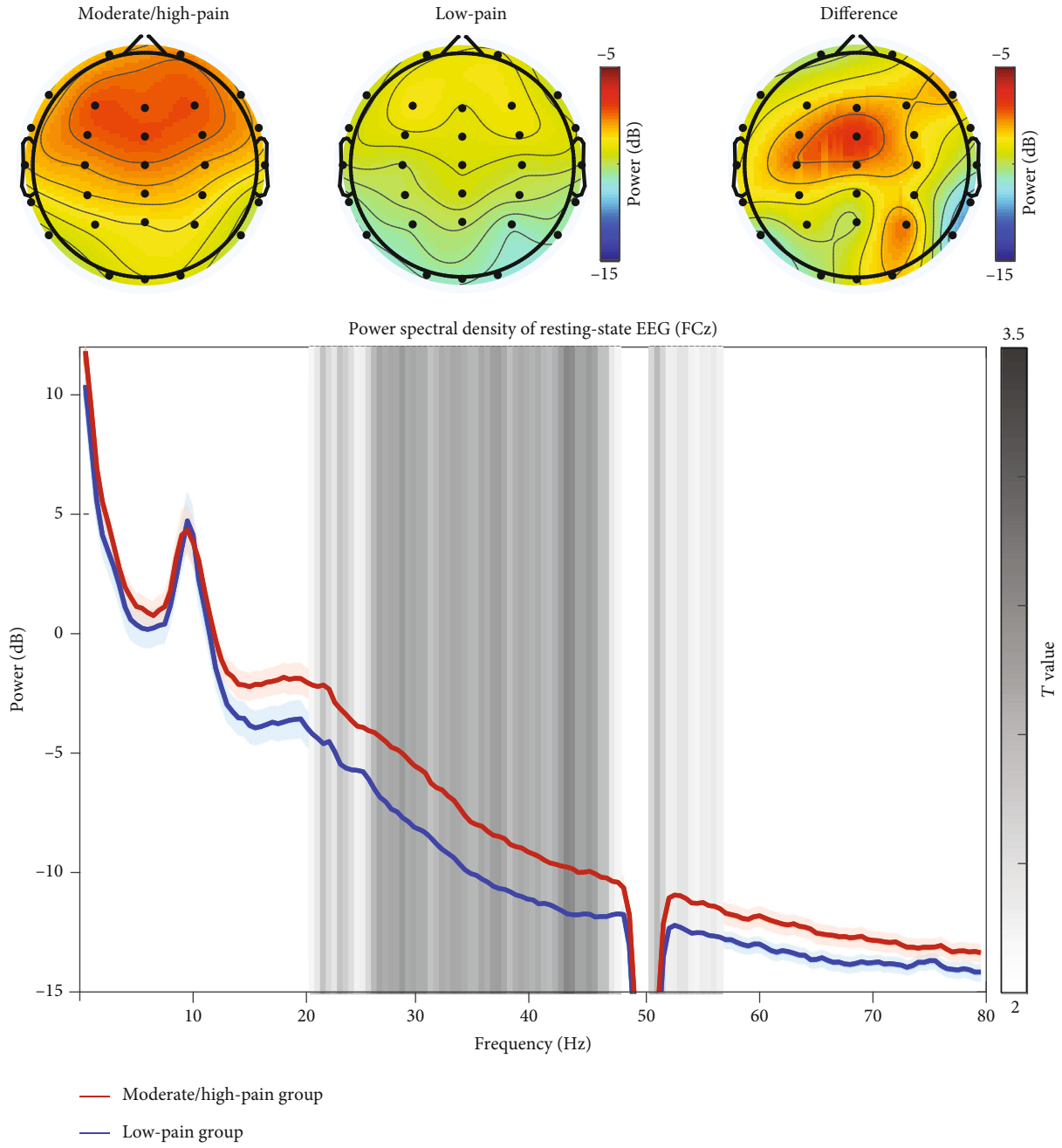

Figure 2: The difference of spectral power density of resting-state neural oscillations between patients with different levels of acute postoperative pain, i.e., moderate/high pain and low pain.

the multivariate machine learning model. The SFFS algorithm was stopped with 50 extracted features for both classification and regression. For classification, LDA with 50 features provided a prediction accuracy of 92.54% in the LOOCV test, i.e., 5 out of 67 patients have been misclassified. For regression, the MLR with 50 features also showed good prediction performance (Figure 5), i.e., the correlation between the real pain intensities and the predicted pain intensities was very strong ($R = 0.84$, $p < 0.001$). These results suggested that the combination of EEG features at the spatial and frequency domains would provide complementary information to achieve better prediction performance than any single EEG feature.

4. Discussion

In the present study, we found that the incidence of moderate-to-high acute postoperative pain after thoracoscopic surgery was high (49%) according to the subjective reports of pain intensity on the 3rd day after the surgery. EEG data collected 2 hours before the surgery showed that patients in the moderate/high-pain group had significantly

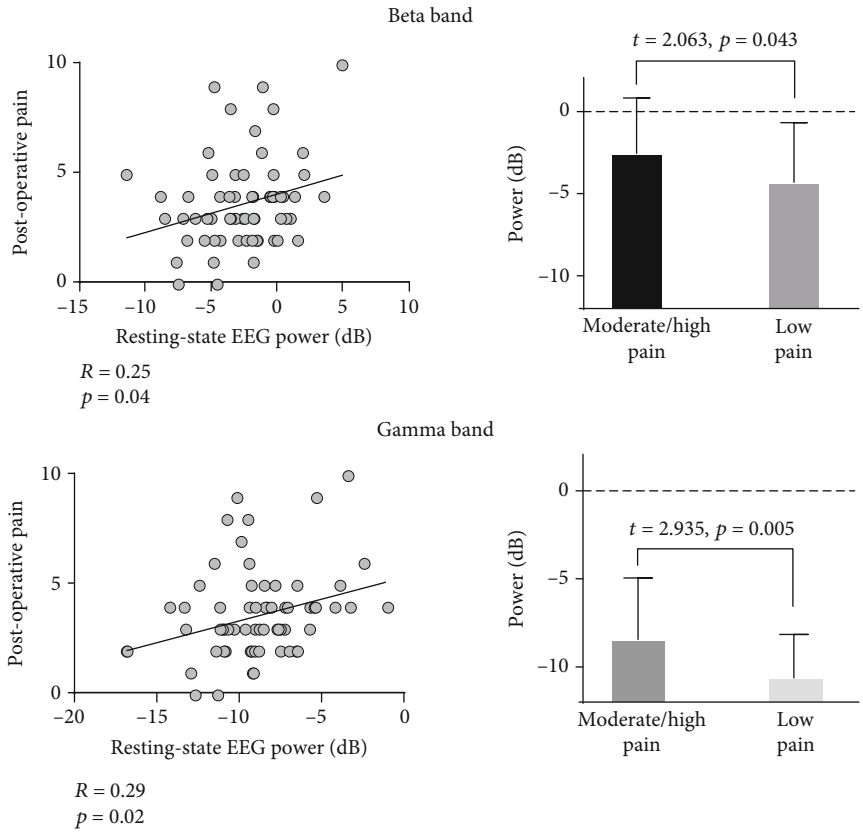

Figure 3: The relationship between postoperative pain and resting-state EEG powers at beta (14-30 Hz) and gamma (31-50 Hz) frequency bands. *Left*: partial correlation was performed after controlling age and removing outliers. *Right*: independent-sample *t*-tests were performed between patients with different levels of acute postoperative pain, i.e., moderate/high pain and low pain.

higher spectral power density of resting-state beta and gamma band oscillations at the frontocentral region than patients in the low-pain group (Figure 2). In addition, a positive correlation was observed between the spectral power density of resting-state beta and gamma band oscillations and subjective reports of postoperative pain (Figure 3). Importantly, applying machine learning technique, the intensity of acute postoperative pain could be accurately predicted based on the recorded resting-state EEG data before the surgery (prediction accuracy is 92.54%, and the correlation coefficient between the real pain intensities and the predicted pain intensities is 0.84). Therefore, our study provided a feasible strategy to identify patients with a high risk of postoperative pain before the surgery using EEG recordings. This strategy could hopefully meet the clinical needs in the future as it would help optimize the analgesic strategy for better control of postoperative pain and other unwanted negative effects.

With the improved understanding of pain analgesic mechanisms, more and more analgesia techniques have been developed to manage postoperative pain [35–38]. Although multimodal analgesia is normally applied in clinical practice, clinical pain assessment based on subjective pain reports, behavioral assessment tools, or solicit input from caregivers [39] suggests that control of postoperative pain is still inadequate [38, 40]. One survey showed that approximately 75% of patients still reported pain after discharge [41]. In the present study, approximately 49% of surgery patients experienced moderate-to-high acute postoperative pain (on the 3rd day after the surgery), for which the incidence of postoperative pain was similarly high in previous studies [40–42]. Please note that significant difference of postoperative pain was already observed on the 2nd day after the surgery between the moderate/high-pain group and the low-pain group. The inadequate management of postoperative pain calls for more effective analgesic strategies. Successful prediction of acute postoperative pain using physiological measures before the surgery would offer valuable information on who needs to be treated preemptively, thus paving the way for more effective and individualized analgesia.

In the present study, the analysis of resting-state EEG data collected 2 hours before the surgery showed that the spectral power density of beta and gamma band oscillations at the frontocentral region was able to distinguish patients with different levels of acute postoperative pain. Additionally, the spectral power density of resting-state beta and gamma band oscillations was positively correlated with the subjective report of postoperative pain.

A series of neural oscillations plays an important role in encoding pain perception for both healthy and disease conditions [8, 15, 16, 43–45]. Evidence showed that beta band oscillations are highly associated with sensorimotor functions, e.g., the preparation before the movement and the calibration during the movement [46, 47]. For instance, beta

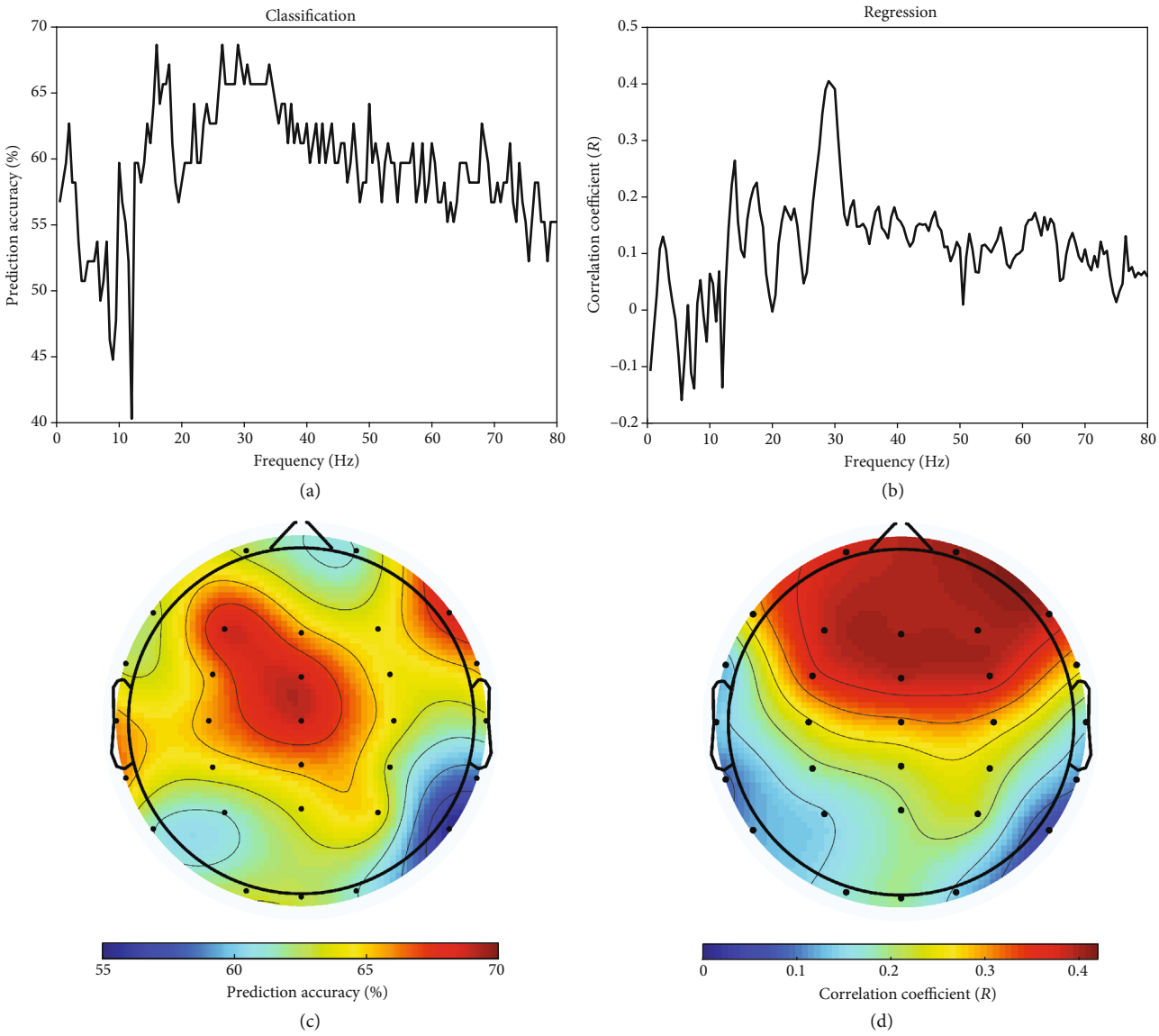

Figure 4: The contribution of presurgery EEG features on the performance of classification and regression to predict the intensity of postoperative pain ((a, b) in the frequency domain; (c, d) in the spatial domain).

band oscillations enhance when the movement is inhibited or voluntarily suppressed [46]. There is a close relationship between movement and pain, and nocifensive behaviors that responded to pain have important protective functions for humans [48]. In addition to movement-related functions, beta band oscillations have been shown to be highly associated with pain in both basic and clinical conditions. In healthy subjects, the power of beta band oscillations has been shown to be modulated by acute pain that was elicited by electrical, laser, and contact heat stimuli [15, 49, 50]. However, it is important to note that the functional interpretation of beta band oscillations is still speculative since, in this study, EEG data were collected before the surgery, and no pain-related movement could be observed at this time. Future studies are required to delineate the detailed mechanism behind the association between the spectral power density of beta band oscillations and acute postoperative pain.

Gamma band oscillations, which are believed to play a crucial role in cortical integration and perception [6, 49, 51], reflect cortical activity directly related to pain perception [16, 51, 52]. For this reason, gamma band oscillations are currently one of the most promising biomarkers of pain perception [5, 51]. Importantly, the solid relationship between gamma band oscillation and pain perception was observed not only at the within-subject level but also across different subjects, in both humans [5, 51] and rodents [14]. In terms of mechanism, the close relationship between gamma band oscillation and pain perception would be associated with the integrating role of gamma band oscillations in the generation of the conscious experience of pain [53], as gamma band oscillations are important for communications within a large network of cortical and subcortical brain regions [17, 54]. Moreover, gamma band oscillations have been demonstrated to subserve a filtering mechanism to select

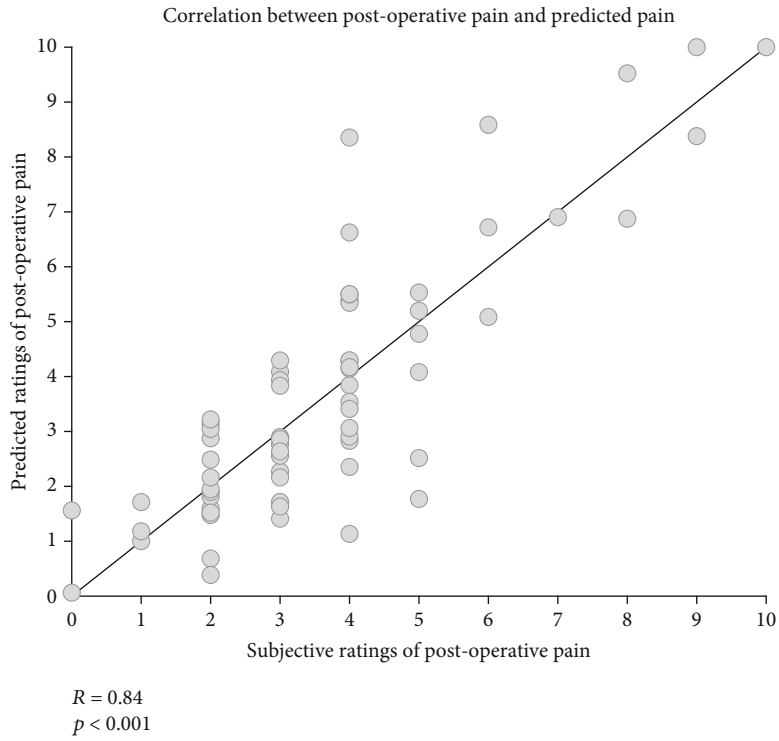

Figure 5: The correlation between subjective ratings of postoperative pain and the predicted ratings of postoperative pain by multiple linear regression with LOOCV.

behaviorally relevant information for action [16, 52]. In the present study, we observed that the spectral power density of gamma band oscillations was positively correlated with the subjective report of postoperative pain. This observation provided additional evidence for the close relationship between gamma band oscillation and pain perception across different human subjects and might be related to the possible communication between distributed neuronal ensembles for subsequent pain-related behaviors [5, 11].

Also, our results showed that pain-related beta and gamma band oscillations were mostly recorded from the frontocentral region. This is reminiscent of previous studies on acute and chronic pain [8, 9, 12, 16]. Some researchers posited that pain chronification involves a shift from brain circuits associated with sensory processes to emotional circuits [55]. Supporting this idea, one previous study showed that beta and gamma band power increases in the dorsolateral prefrontal and orbitofrontal cortex were correlated with higher affective pain scores in fibromyalgia patients [8]. In addition, the prefrontal cortex is generally believed to be involved in emotion processing [56]. The tonic and ongoing nature of clinical pain resembles that of chronic pain better than phasic pain, like transient laser-induced thermal pain, in which the somatosensory cortex seems to play crucial roles [5]. The importance of prefrontal oscillations, therefore, may suggest that the emotional processing in patients who tend to develop postoperative pain is already somewhat compromised in the presurgery stage, even though these patients may display no explicit emotional disorders or manifestly abnormal emotional processing. Future studies are needed to test whether emotion does play some roles in the relationship between neural oscillations and the probability of developing postoperative pain. It should be noted that no significant correlation between alpha oscillations and acute postoperative pain was observed in the present study. Since alpha oscillations are highly sensitive to the state of the patients (e.g., attention and anticipation), it might be possible that alpha oscillations could be able to predict the intensity of pain perception within a short period of time (e.g., several seconds) [17], but not within a long period of time (e.g., several days in the present study).

The relationship between neural oscillations and acute postoperative pain provided a solid basis for the prediction of postoperative pain with physiological measures before the surgery. The use of machine learning techniques (i.e., LDA with SFFS) achieved a prediction accuracy of 92.54% and a correlation coefficient of 0.84 based on resting-state EEG activities that were recorded 2 hours before the surgery. Ideally, the combination of resting-state EEG recording technique and machine learning algorithms would yield diagnostic biomarkers of acute postoperative pain. This diagnostic biomarker would be important for the development of effective analgesic strategies in the perioperative period, which would be helpful in controlling postoperative pain in surgery patients [21, 22, 53]. In practice, the EEG device is portable and widely equipped, which enables the feasibility in most clinical situations for the application of an EEG-based diagnostic biomarker. Nevertheless, this study did not detangle the respective contribution of trait and state characteristics of patients to the prediction success since EEG data collected 2 hours before the surgery may reflect both characteristics. Importantly, both of them could contribute to the individual

difference in analgesic efficacy. If it were trait characteristics that mattered, specifically targeting patients with those traits may be more beneficial and cost-effective. If it were state characteristics, interventions manipulating those states might help achieve better analgesic efficacy.

Several additional limitations in the present study should be noted. First, the current clinical experimental design would possibly be confounded by some unwanted factors. Although a standardized operative procedure was adopted for all patients, there were some variations during the surgery (e.g., the duration and complexity of the surgery and the types and amounts of analgesics), which might also affect the acute postoperative pain. Second, the sample size of the present study is limited, and the age, the educational level, and the type of surgery varied a lot among subjects. In addition, more patients scheduled for other types of surgery should be considered in future studies to verify the identified EEG-based biomarker of postoperative pain. Third, this study did not testify the specificity of EEG oscillations to acute pain. Some studies suggest that gamma oscillations selectively encode phasic and tonic thermal pain [5, 16], but this issue is still in a heated dispute. More importantly, this study did not explicitly address this issue, even though neural oscillations were demonstrated to predict postoperative pain. Future research may test the specificity of EEG oscillations and offer further insights into specifically predicting postoperative pain with neural oscillations or other physiological measures.

In conclusion, we provided solid evidence for the close relationship between the spectral power density of resting-state beta and gamma band oscillations at the frontocentral region and subjective reports of postoperative pain. Moreover, we predicted the level of acute postoperative pain based on features of neural oscillations using machine learning techniques, which achieved a prediction accuracy of 92.54% and a correlation coefficient between the real pain intensities and the predicted pain intensities of 0.84. Therefore, the prediction of acute postoperative pain based on neural oscillations measured before the surgery is feasible and could hopefully meet the clinical needs in the future, thus helping optimize the analgesic strategy for better control of postoperative pain and other unwanted negative effects.

Data Availability

Email to doctor_yifeng@sina.com.

Conflicts of Interest

All authors declare no competing interests.

Authors' Contributions

Fei Gao, Qi Han, and Yi Feng were responsible for the study design. Fei Gao, Qi Han, and Yi Feng were responsible for the protocol design. Yi Feng was the advisor for study protocol and management of the study. Qi Han was responsible for patient recruitment. Qi Han and Yi Feng were responsible for data collection. Fei Gao, Qi Han, and Yi Feng were responsible for study conduct. Yi Feng and Li Hu were responsible for study monitoring. Lupeng Yue, Gao Fei, Qi Han, and Li Hu were responsible for data analysis. Lupeng Yue, Qi Han, and Li Hu were responsible for data evaluation. Lupeng Yue, Qi Han, Libo Zhang, and Li Hu were responsible for writing the manuscript. Qi Han, Lupeng Yue, Gao Fei, Libo Zhang, Yi Feng, and Li Hu were responsible for editing and approval of the manuscript. Q. H. and L. Y. contributed equally. Y. F. and L. H. contributed equally.

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Review Article

The Distinct Functions of Dopaminergic Receptors on Pain Modulation: A Narrative Review

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Chronic pain is considered an economic burden on society as it often results in disability, job loss, and early retirement. Opioids are the most common analgesics prescribed for the management of moderate to severe pain. However, chronic exposure to these drugs can result in opioid tolerance and opioid-induced hyperalgesia. On pain modulation strategies, exploiting the multitarget drugs with the ability of the superadditive or synergistic interactions attracts more attention. In the present report, we have reviewed the analgesic effects of different dopamine receptors, particularly D1 and D2 receptors, in different regions of the central nervous system, including the spinal cord, striatum, nucleus accumbens (NAc), and periaqueductal gray (PAG). According to the evidence, these regions are not only involved in pain modulation but also express a high density of DA receptors. The findings can be categorized as follows: (1) D2-like receptors may exert a higher analgesic potency, but D1-like receptors act in different manners across several mechanisms in the mentioned regions; (2) in the spinal cord and striatum, antinociception of DA is mainly mediated by D2-like receptors, while in the NAc and PAG, both D1- and D2-like receptors are involved as analgesic targets; and (3) D2-like receptor agonists can act as adjuvants of μ-opioid receptor agonists to potentiate analgesic effects and provide a better approach to pain relief.

1. Introduction

Recently, the definition of pain has been revised by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [1, 2]. Vital to survival, pain functions as a protective alarm for an organism to identify and avoid possible life-threatening situations [3]. When pain lasts beyond normal tissue healing time (i.e., more than three months), it is known as chronic pain: a pathological condition with dramatic costs and suffering [4–6]. Pain is the integration of nociception with consciousness, feeling, and emotion and differs from nociception. It relies on the peripheral signaling pathway and involves several regions of the brain, including the thalamus, medial prefrontal cortex (mPFC), nucleus accumbens (NAc), periaqueductal gray (PAG), insula, somatosensory cortex, amygdala, and striatum [7–9].

The most widely prescribed analgesic drugs for pain relief, such as nonsteroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase-2 (COX-2) inhibitors, and opioids, may have some adverse effects [10, 11]. For instance, a number of reports have proven that NSAIDs increase the risk of severe bleeding (especially gastrointestinal bleeding) and cardiovascular diseases (such as myocardial infarction and strokes), resulting in a higher rate of mortality [12, 13]. COX-2 selective inhibitors like celecoxib and rofecoxib show equivalent or superior analgesic and anti-inflammatory effects compared to conventional NSAIDs and carry a lower risk of gastrointestinal bleeding [14]. However, it has been demonstrated in animal models that inhibition of COX-2 activity might suppress bone healing [15, 16]. Although among analgesic agents, opioids are the most potent drugs for treating severe pain (e.g., cancer and perioperative pain), the efficiency of their long-term use for chronic pain is controversial [17, 18]. The critical problem is that long-term

administration of opioids can cause addictive behaviors in patients (22.6%), further contributing to the opioid crisis [19, 20]. This evidence indicates that it is essential and urgent to investigate the analgesic efficacy of other known drugs or to develop new analgesic drugs with reduced side effects and abuse liability that target novel pathways.

A growing body of evidence from preclinical and clinical studies suggests that the dopamine (DA) system contributes to the pathology of the most chronic pain conditions [21, 22]. For instance, the studies with functional magnetic resonance imaging (fMRI) technique provided solid evidence suggesting that the mesolimbic dopamine circuit and pain modulation system are largely overlapped, including the ventral tegmental area (VTA), prefrontal cortex (PFC), amygdala, and nucleus accumbens (NAc) [23–25]. The functional connectivity between the aforementioned brain regions and their gray matter volumes was significantly different between chronic pain patients and healthy controls [26, 27]. And positron emission tomography (PET) used in the evaluation of patients with fibromyalgia syndrome demonstrated that synthesis and release of DA reduced in the presynaptic neurons [28]. Similar results were found in patients with back pain, indicating that altered brain DA function was associated with pain sensitivity and the affective state [29]. Besides this, several clinical studies have shown that the administration of levodopa, the precursor of DA, alleviates pain associated with Parkinson's disease in humans [30–32]. In animal models, injection of levodopa into the intrathecal (IT) space or some regions of the central nervous system (such as the NAc, dorsolateral striatum, and prefrontal cortex) had adequate analgesic effects [33, 34]. In general, these results indicate that the regulation of the dopaminergic system may be a novel strategy to manage pain effectively.

DA mainly acts on two subfamilies of DA receptors: D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptors [35]. This classification is based on the biochemical and pharmacological properties of the receptors [36]. D1 subfamily receptors are coupled to G-protein alpha subunits ($G_{\alpha s}$ and $G_{\alpha olf}$), induce $3',5'$-cyclic adenosine monophosphate (cAMP) production, and activate protein kinase A (PKA) [37]. Members of the D2 subfamily are generally coupled to $G_{\alpha i}$ and $G_{\alpha o}$ subunits, inhibit the activity of adenylate cyclase, reduce the production of cAMP, and, subsequently, reduce PKA activity [38]. Although DA receptors are widely distributed in the brain, the densities of the receptor subtypes vary between different areas [39]. The D1 receptor is the most widespread protein in rat brains, whereas the D2 receptor is mainly distributed in specific regions such as the striatum, NAc, and some limbic regions [36, 40].

Previous reports have proven the role of the hypothalamic-spinal DAergic system on pain modulation, suggesting that the antinociceptive effects of DA are mediated by D2-like receptors, while the pronociceptive effects are mediated by D1-like receptors [41]. However, the available data fail to definitively elucidate the role of D1-like receptors on pain modulation and the effect of the central DAergic system on pain processing [41]. In the present review, we more comprehensively investigated the roles of DA receptor subtypes on pain modulation, especially D1 and D2 receptors in the central nervous system (CNS), to illustrate their potential analgesic features.

2. Spinal Cord

The spinal cord is the first relay station in the transmission of nociceptive information from the periphery to the brain. It has been proven that all types of DA receptors are present in the primary nociceptors and different laminars of the dorsal horns of the spinal cord and that D2 receptors are the most abundantly expressed [41, 42]. This indicates that DA functions in the modulation of pain signals by affecting presynaptic and postsynaptic neurons [41]. Numerous studies illuminated the contributions of D1- and D2-like receptors in the spinal cord during pain modulation. In cases of acute pain, intrathecal (IT) administration of DA or quinpirole (a D2/3 receptor agonist) was reported to increase the mechanical pain threshold, measured using a von Frey anesthesiometer. This analgesic effect was reversed by IT coadministration of D2, D3, and D4 antagonists [43, 44]. It was also demonstrated that IT administration of apomorphine or DA could enhance the thermal threshold, which was estimated by the tail-flick test [45–47] and the hot plate test [47]. These features were mimicked by administration of LY171555 (a D2-like receptor agonist), but not SKF-38393 (a D1/D5 receptor agonist), and inversed by D2 antagonists (e.g., *cis*-flupenthixol and sulpiride) [47]. For inflammatory pain induced by carrageenan, IT administration of LY171555, but not SKF-38393, rescued the thermal withdrawal latency (measured with Hargreaves apparatus) [48]. Similar findings were obtained from a neuropathic pain model of chronic constriction injury (CCI) of the sciatic nerve in which IT administration of levodopa decreased tactile and cold allodynia. This effect was blocked by sulpiride [34]. Furthermore, in a follow-up study, the same group found that lumbar IT injection of quinpirole produced short-term inhibition of responses to cold and tactile stimuli, which coincided with pain relief by levodopa administration in neuropathic pain [49].

Because DA can affect both exploratory and goal-oriented movements, the findings of these studies on the analgesic effects of DAergic drugs should be carefully interpreted [50]. These effects could potentially interfere with the results of pain-related behavioral tests, since the majority of measurements of the pain threshold utilize a withdrawal movement to a nociceptive stimulus [51]. For example, an effective analgesic dose of quinpirole administered systemically was proven to affect locomotion and exploratory activities of rats in an open-field maze [33]. While the intracisternal injection of quinpirole could successfully relieve formalin- and capsaicin-evoked trigeminal pain, a higher dose of quinpirole (20 nmol) had no effect on motor performance in the rotarod test (commonly used to evaluate motor coordination) [52]. Briefly, systematic administration of a high dosage of quinpirole may have affected the locomotor and exploratory activities of rats, while IT infusion of low doses had no significant effect on locomotion. Besides, the effects of D2 receptor agonists on locomotion might be strain-dependent, as shown in spontaneously hypertensive

rat (SHR) and SLA16 isogenic (SHR.Lewis-*Anxrr16*; anxiety-related response #16) rat strains [53]. In summary, the dosage of drugs, route of administration, and strain of animals should be considered when investigating the possible analgesic effects of DAergic drugs.

In vitro studies have provided compelling evidence that DA is involved in pain signaling by modulation of intrinsic excitability and synaptic transmission of dorsal root ganglion neurons and spinal neurons [54]. For the first time, Tamae et al. used the whole-cell patch-clamp technique to record the excitability of spinal dorsal horn neurons and study the effects of DA receptor agonists. They found that bath application of DA hyperpolarized the membrane potential of substantia gelatinosa neurons and suppressed electrical stimulation action potential in the dorsal root. Quinpirole simulated a DA-induced outward current, which was not produced by SKF-38393 [43]. Moreover, activated D2 receptors in neurons of the superficial medullary dorsal horn inhibited the C-fiber-evoked action potentials of wide dynamic range neurons in the trigeminal spinal nucleus [52]. Consistent results were revealed in neuropathic pain, which reported that C-fiber-evoked action potentials in the spinal dorsal horn were dose-dependently ameliorated by spinal superfusion of quinpirole in both nerve-injured and sham-operated rats [55]. Collectively, D2 receptors may play an analgesic role in the spinal cord by reducing the amount of sensory inputs from nociceptors to the CNS (Table 1).

Interestingly, D1-like receptor agonists (SKF-83959 and SKF-81297) mimic the inhibitory effects of DA on slow ventral root potentials, which is attributed to responses evoked by C-fibers that reflect nociceptive transmission in the spinal cord. In addition, the inhibitory effects were attenuated by D1-like receptor antagonists (SCH-23390 and LE300) [56]. Taken together, there is widespread disagreement among *in vitro* studies about the role of D1-like receptors. However, *in vivo* investigations found that the D1 receptor had no significant analgesic effect in the spinal cord. These findings could be related to the lower affinity of DA for D1 receptors. Thus, only a few reports found that the activation of D1 receptors in the spinal cord could mimic the role of dopamine, while, in *in vivo* studies, not even a faint effect could be observed in behavioral testing.

3. Brain

The brain contains a high density of DA receptors in regions that functionally contribute to integrating the consciousness and emotion of pain, such as the ventral tegmental area (VTA), mPFC, NAc, PAG, and striatum [57, 58].

3.1. Ventral Tegmental Area (VTA). The VTA, a part of the DAergic system, is composed of mesolimbic DA neurons that project to the NAc and mPFC [59, 60]. It is involved in various physiological functions such as pain processing and motivation [59]. For instance, the excitability of VTA DA neurons decreased significantly in CCI mice models. Optogenetic stimulation of VTA DA neurons produced analgesic effects [61]. In another study, voluntary wheel running was shown to produce hypoalgesia by reversing the inactivation of VTA DA neurons in a rat model with neuropathic pain [62]. Noxious footshocks are believed to inhibit the activities of DA neurons in the dorsal VTA, but physically excite the activities of DA neurons in the ventral VTA, suggesting that VTA DA neurons are heterogeneous in the processing of rewarding and aversive events [63]. Furthermore, a few reports have focused on the roles of different DA receptors in pain modulation. Pretreatment of VTA with both sulpiride [64] and SCH-23390 [65] could inhibit antinociception induced by intralateral hypothalamus (LH) microinjection of carbachol, obtained by the tail-flick test. The precise function of VTA DA neurons in the pain process is still unclear.

3.2. Nucleus Accumbens (NAc). Functionally, NAc contributes to a wide range of reward-related behaviors [66]. As one of the two main projection target regions of VTA DAergic neurons (the other being the mPFC) [67], the NAc is a rostral telencephalic gray mass with a heterogeneous structure. Anatomically, it can be divided into the shell and core regions [68]. The shell is the outer region innervated by DAergic neurons and is closely related to the mesolimbic system. It has been identified as playing a profound role in motivation and pain modulation pathways [60, 69]. In contrast, the NAc core region, which connects to the caudate-putamen and striatum, is involved in goal-directed behaviors [70, 71]. Both D1 and D2 receptors were reported to be expressed in the whole NAc, while D3 receptors are selectively expressed in the shell region [69]. The past few decades have witnessed a drastic rise in the number of studies attempting to identify the analgesic effects of DA receptors in the NAc region. For example, stimulation of D2 receptors in the NAc was revealed to inhibit the persistent phase of formalin-induced nociception. The recommended dose of quinpirole did not produce overt behavioral changes, as shown in the rotarod treadmill test. However, the D1-selective agonist SKF-38393 had no significant effect on the nociceptive behavior induced by formalin [70]. Even so, a number of reports have demonstrated that blocking both D1- and D2-like receptors of the NAc attenuated analgesia induced by forced swim stress [72] and carbachol injection into the LH [73] in the formalin test, particularly in the late phase. And intra-accumbal administration of SCH-23390 and sulpiride dose-dependently prevented intra-VTA orexin-induced antinociception measured by the tail-flick test [74]. In addition, mRNA levels of D2 and D1 receptors both decreased in the NAc of animals with neuropathic injury, including spared nerve injury [75] and CCI [76].

Although the analgesic effects of D2 receptors in the NAc are more profound, blocking both D1- and D2-like DA receptors showed similar pharmacological effects [71, 72, 74]. The similarity of D1 and D2 receptors in the NAc may be attributed to their distinct DAergic innervations. The first relates to rewarding and pleasurable effects that act on primary D1 receptors of spiny neurons via direct pathways, and the second relates to aversive and negative effects that indirectly operate on spiny neurons, which is diminished by the activation of D2 receptors [77]. In other words, the activation of D1 receptors in the NAc probably weakens pain by enhancing the reward and pleasure effects, while D2

Table 1: The role of DA receptors on pain modulation in the spinal cord.

Authors	Drugs	Models	Measurements	Main results
Almanza et al. [44]	Quinpirole (D2/3 agonist)	Acute pain	Von Frey Hargreaves apparatus	(i) The activation of dopamine D2 receptors increased mechanical threshold
Barasi and Duggal [45] Jensen and Smith [46] Liu et al. [47]	LY171555 (D2 agonist) SKF38393 (D1/D5 agonist)	Acute pain	Tail-flick test Hot plate test	(i) The D2 agonist mimicked the analgesic effect of DA, but the D1 agonist did not
Gao et al. [48]	LY171555 SKF38393	Inflammatory pain induced by carrageenan	Hargreaves apparatus	(i) The D2 agonist rescued the thermal withdrawal latency, but the D1 agonist did not
Cobacho et al. [49]	Levodopa Sulpiride (D2 antagonist)	Neuropathic pain induced by chronic constriction injury	Tactile and cold allodynia test	(i) Levodopa decreased the tactile and cold allodynia, which was blocked by the D2 antagonist
Tamae et al. [43]	Quinpirole SKF 38393	Acute pain	Von Frey filament whole-cell patch-clamp technique	(i) The D2 agonist simulated the analgesic effect of DA at both behavioral and electrophysiological levels, but the D1 agonist did not
Lapirot et al. [52]	Quinpirole Sulpiride	Acute pain	Unitary extracellular recordings Facial capsaicin and formalin test	(i) The activation of D2 receptors inhibited both formalin- and capsaicin-evoked pain behaviors and the C-fiber-evoked action potential firing

activation may reduce aversion to pain [78]. Compared to D1-like receptors in the NAc, D2-like receptors have a higher affinity to DA and are activated preferentially [79, 80]. However, further studies are needed to shed light on the potential mechanisms of D1- and D2-like receptors in pain modulation.

3.3. Prefrontal Cortex (PFC). Studies over the past two decades have revealed the prominent role of the PFC in reward and pain modulation [81, 82]. As a region of the cerebral cortex located at the front of the frontal lobe, the PFC is a key structure contributing to critical brain functions such as memory, learning, attention, problem-solving, planning, and social behavior [83]. Recently, the important role of the DAergic signaling pathway from the VTA to the PFC on pain modulation has been proven [60, 84]. Anatomically, the PFC can be divided into the medial PFC (mPFC), dorsolateral PFC (dlPFC), ventrolateral PFC (vlPFC), orbitofrontal cortex, and caudal PFC [85]. Due to the potential pain modulating mechanisms of the DA system in the PFC, neuronal responses of the PFC were recorded using an extracellular recording unit in urethane-anesthetized rats. Applying a high-frequency stimulation (50 Hz, 250 μA, 100 μs square pluses, 30 s) to the VTA by a tungsten microelectrode (impedance 8-12 MΩ) suppressed nociceptive responses for an extended period in the rat PFC. Similarly, injection of a selective D2 receptor into the rat PFC produced long-lasting suppression of nociceptive responses, which was reversed by the blockade of D2 receptors. In contrast, the D1 antagonist/agonist was minimally effective in nociceptive responses [84].

The mPFC has a critical role in both reward and pain processing [86]. Projection of adrenergic neurons from VTA to mPFC (the DA inputs from VTA to mPFC) regulates the neural functions of mPFC (e.g., executive activities, excitability, and synaptic transmission) [87]. In chronic pain rodent models, the activity of neurons in the mPFC was reportedly reduced [88]. Optogenetic studies revealed that the activation of DA signaling from the VTA into the mPFC modulated hypersensitivity in mice with spared nerve injury neuropathic pain [89]. In summary, these studies suggest that the DA system in the mPFC may be a target for the relief of chronic pain.

DA modulation in the mPFC is also related to attention tasks for this region. Previous studies have proven the impairment of memory and attention by chronic pain [90]. In a rodent model, blocking the activity of D1 receptors in the mPFC impaired the attentional set-shifting task, whereas enhancing D1 receptor activity improved this performance [91, 92]. Based on these findings, clinical studies have shown that distraction tasks could decrease pain perception and relieve chronic back pain [93, 94]. Together, pain modulation of DA receptors in the mPFC may underlie distinct manners: D1 receptors tend to affect the cognitive aspect of pain (e.g., by regulating distraction), while D2 receptors may directly regulate the pain perception or nociceptive responses [83, 95]. Because of the complexity of the neuronal network, cellular receptor expression, DA concentration, and receptor affinity, it remains unclear as to how the DA system in the mPFC is involved in the pain modulation process.

3.4. Striatum. The dorsal striatum, which receives afferents from the sensorimotor cortex and DA-containing inputs

Table 2: The role of DA receptors on pain modulation in the brain.

Authors	Brain regions	Models	Drugs	Measurements	Main results
Moradi et al. [64]	VTA	Acute pain	D1 antagonist SCH23390 D2 antagonist sulpiride	Tail-flick test	(i) Blockage of the D1 and D2 receptors had a similar effect, as inhibiting the antinociception induced by carbachol
Taylor et al. [70] Siahposht-Khachaki et al. [73] Yazdi-Ravandi et al. [74] Martikainen et al. [29]	NAc	Acute pain and neuropathic injury (SNI and CCI)	D1 antagonist SCH23390 D1 agonist SKF38393 D2 antagonist sulpiride D2 agonist quinpirole	Formalin test Tail-flick test	(i) Neuropathic injury decreased the mRNA levels of both D2 and D1 receptors (ii) The blockade of D1- and D2-like receptors showed the similar pharmacological effects (iii) The activation of the D2 receptors had more profound analgesic effects
Sogabe et al. [84] Granon et al. [92] Fletcher et al. [91]	PFC	Acute pain In vitro	The same as above	Electrophysiological recording Attentional set-shifting task	(i) The D1 receptors tend to affect the cognitive aspect of pain, like via distraction (ii) The D2 receptors may directly regulate the pain perception or nociceptive responses (iii) The activation of the D2 receptors had more profound analgesic effects
Magnusson and Fisher [98] Ansah et al. [99] Saunier-Rebori and Pazo [101]	Striatum	Inflammatory pain	D1 antagonist SCH23390 D1 agonist SKF38393 D2 antagonist eticlopride D2 agonist quinpirole	Formalin test Jaw opening reflex	(i) The activation of the D2 receptors had antihypersensitive effect, but not D1 receptors
Hagelberg et al. [103, 104]	Striatum	Clinical chronic orofacial pain		PET	(i) Healthy male volunteers with a low D2 receptor, while D2 receptor availability of patients increased, which predicted a low synaptic DA concentration and a high capacity of recruiting pain inhibitory circuitry
Ben-Haim et al. [109] Li et al. [107] Tobaldini et al. [114]	PAG	Acute pain	DA agonist apomorphine D2 antagonist eticlopride D1 antagonist SCH-23390 Opiate heroin and morphine	Hot plate test Tail-flick test Mechanical paw-withdrawal test	(i) The analgesic effect of apomorphine was blocked by D2 antagonist, but not by D1 antagonist (ii) Blockage of the D1 and D2 receptors had a similar effect, as reducing the antinociception induced by the opioids (iii) The activation of D1 receptors could enhance the opioid- and D2-induced antinociception

from the pars compacta of the substantia nigra, has abundant D1 and D2 receptor expressions [96, 97]. Microinjection of a D1 antagonist (SCH-23390) or a D1 agonist (SKF-38393) into the dorsolateral striatum was reportedly not effective in formalin-induced nociception. Conversely, a D2 antagonist (eticlopride) could enhance the formalin-induced nociception, while a D2 agonist (quinpirole) exerted an opposite effect [98]. A similar result was found in neuropathic conditions in rats with unilateral ligation of the tibial and common peroneal nerves [99]. The antihypersensitive effect is induced by striatal D2 receptors and involved in inhibiting the impulse discharge of, presumably, pronociceptive neurons in the rostral ventromedial medulla, which is an important structure in descending pain modulation [100]. The antihypersensitive effect can be reversed by spinal administration of a D2 receptor antagonist [99]. Microinjection of quinpirole into the striatum inhibited the jaw opening reflex (JOR, an indicator of pain), evoked by tooth pulp stimulation, in a dose-dependent manner. This effect was blocked by haloperidol (a D2 receptor antagonist), whereas SKF-38393 left the JOR amplitude unaffected. Intrastriatal microinjection of quinpirole significantly reduced the responses of Aβ-

and C-fiber afferents associated with inhibition of the JOR [101]. Thus, it is possible that striatal D2 receptors attenuate pain-related responses through final descending sensory pathways.

Notably, evidence from human studies also indicated that striatal D2 receptors are involved in the regulation of pain [102]. A previous PET study showed that healthy male volunteers with low D2 receptor availability in the right putamen exhibited a high cold-pain threshold, while a low heat-pain modulatory capacity was associated with low D2 receptor availability in the left putamen [103]. Similarly, D2 receptor availability in the left putamen of patients with chronic orofacial pain increased [104]. Such results concur with experimental animal studies. More specifically, a high level of synaptic DA can lead to low availability of D2 receptors, a high cold-pain threshold, and a low response capacity to conditioning stimulation [103]. In other words, the high availability of D2/D3 receptors predicts a low synaptic DA concentration and a high capacity of recruiting pain inhibitory circuitry [96]. In addition, previous reports also demonstrated that striatal D2 receptors may influence pain-related responses not only through descending modulation of sensory pathways but also through supraspinal action on nonsensory factors [96, 97, 102]. For instance, the responses of the subject's attitude toward pain were negatively correlated with baseline striatal D2/D3 receptor availability [105]. Thus, subjects with high availability of striatal D2/D3 receptors, probably indicating low extracellular endogenous DA levels, rate the same stimulus as more painful than subjects with low striatal D2/D3 receptor availability [106].

3.5. Periaqueductal Gray (PAG). The midbrain PAG, a region full of opioid receptors, plays a significant role in the modulation of nociception. A subpopulation of DAergic neurons (A10-dc) known to be involved in the modulation of endogenous pain exists in the ventrolateral PAG (vlPAG), which projects locally within the PAG or to forebrain regions [107, 108]. Electrical stimulation of the PAG led to pain relief in animal models [109, 110]. Also, impairment of dopaminergic neurons of the PAG reduced antinociception induced by opioids [111]. In another study, enhanced levels of tyrosine hydroxylase following induction of CCI resulted in an enhanced level of DA in the PAG [112].

Microinjection of (–) apomorphine (DA agonist) into the ventral PAG (vPAG) was shown to increase the latency to lick the hind paw in the hot plate test in a dose-dependent manner. This effect was blocked by pretreatment with eticlopride (D2 antagonist), but not SCH-23390 (D1 antagonist). Apomorphine infusion into the outside of the vPAG had no marked analgesic effect [113]. In contrast, the findings of another study showed that administration of D1 receptor antagonist SCH-23390 in PAG dose-dependently attenuated analgesia induced by opiates (e.g., heroin and morphine). The effect was observed by the behaviors integrated supraspinal response (examined by hot plate test), but not the simple spinal reflex (examined by the tail-immersion test) [111]. Additionally, several studies have found that injection of both D2-like and D1-like receptor antagonists (raclopride and SCH-23390, respectively) into the PAG reduced antinociception induced by the activation of μ-opioid receptors [114]. Selective activation of D2-like receptors within the PAG significantly reduced allodynia, which was also blocked by GABAA receptor agonist (muscimol), opioid receptor antagonist (naloxone), and D2-like receptor antagonist (raclopride). Although the analgesic effect induced by activating D1-like receptors in the PAG was tiny and transient, it enhanced the antinociceptive effects of D2-like receptors [114]. Notably, treatments with all drugs had no significant influence on the locomotion of rats observed in open-field tests. Overall, these findings indicate that the activation of D2 receptors may induce the antinociceptive effects directly, while the activation of D1 receptors may participate synergically in the opioid-induced and D2-induced antinociception [111, 114].

In short, D2-like receptors may exert a higher analgesic potency, but D1-like receptors act in different manners with distinct mechanisms in the mentioned regions (Table 2). However, more studies should be designed to thoroughly investigate the role of DA receptors in antinociception and the underlying mechanisms.

4. Discussion and Conclusions

Complaints about acute and chronic pain reflect that pain management is poorly served by existing treatments. Developing novel analgesics with superior efficacy, diminished adverse effects, and lower abuse liability is urgent. Previous studies showed that the DAergic system plays a critical role in pain modulation [21, 115], suggesting that targeting DA receptors may be a novel treatment strategy for chronic pain. Therefore, we reviewed the role of DA receptor subtypes in the pain processes, particularly D1 and D2 receptors, throughout the central nervous system.

The evidence from studies on the spinal cord and brain cortex consistently indicated that more potent analgesic effects are related to D2 receptors, while the role of D1 receptors on pain modulation varies between different parts of the nervous system. Firstly, D1 receptors are mainly located postsynaptically, while D2 receptors are in both post- and presynaptic regions. Presynaptic DA receptors are characterized by higher sensitivity (5- to 100-fold) than those located in postsynaptic parts [116]. Therefore, compared to the D1 receptors, D2 receptors have a higher affinity for DA and require lower concentrations of DA to be activated. Secondly, D2 receptor signaling has an inhibitory effect, and the activation of D2 receptors decreases a neuron's firing rate, resulting in the network requiring more stimuli to initiate DA transmission [21]. For instance, in the spinal cord, D2 receptors play an analgesic role by reducing the intensity of the sensory input, like depressed C-fiber-evoked field potentials [55]. However, D1 receptors mediate neuronal excitation, which relies on the complex neuronal network between regions and a higher DA level.

In addition to the more potent analgesic efficacy of D2 receptors, D1 family receptors may play a more important role than D2 family receptors in mediating the facilitation of abuse-related intracranial self-stimulation (ICSS; one experimental procedure evaluated abuse-related effects of

drugs) [117]. High-efficacy D1 agonists SKF-82958 and A77636 produced facilitation of abuse-related ICSS depending on dosage and time. Lower efficacy D1 ligands and all D2/3 ligands failed to facilitate ICSS at any dose or pretreatment time. Besides, D2 receptor agonists are long prescribed and well tolerated, which are excellent features of clinical medicine [55].

Notably, although mounting evidence has revealed the antinociceptive effects of D2-like receptors, their analgesic efficacy is not as high as other drugs [55, 115]. Fortunately, a few studies have suggested that D2 receptors establish complex interactions with opioid receptors and could potentiate the analgesic effects of μ-opioid receptor agonists. For instance, coadministration of 1 μmol/L through spinal infusion was insufficient to alter potentials evoked by electrical C-fiber stimulation but could dramatically enhance the potential inhibition effects of DAMGO ([D-Ala^2,N-MePhe^4,Gly-ol]-enkephalin; a μ-opioid receptor agonist) and reduced its half-maximal inhibitory concentration (IC50) by 2-fold in a rat model of peripheral nerve injury [55]. Furthermore, the IT administration of quinpirole in lower doses remarkably augmented the antinociception effects of DAMGO (8-fold) in both inflammatory and neuropathic models of pain, assessed by mechanonociception and thermonociception behavioral tests [118]. Systemic administration of *R*-VK4-40, a highly selective D3 receptor antagonist, produced mild antinociceptive effects without altering locomotion, as observed in the open-field test or rotarod locomotor performance. In rats, this substance diminished the rewarding potency of oxycodone (the most commonly abused prescription opioid) and mitigated its tolerance and dependence without compromising its analgesic effects [119]. Thus, targeting the D2-like receptors may have the potential to enhance the analgesic property and alleviate the adverse effects of opioids.

Moreover, as previous reviews focused more on the role of the two subfamilies of DA receptors in the spinal cord and hypothalamus, the present review provides a more comprehensive summary on this topic by including the spinal cord and different brain regions. Indeed, some other regions (e.g., the hippocampus and amygdala), which are also involved in pain modulation, were excluded from the review due to the shortage of relevant studies, making it difficult to deduce a reliable conclusion. Moreover, even though we summarized the mutual promotion between the D2 receptor agonists and the opioids, whether chronic exposure to coadministration of D2-like receptor agonists and opioids could decrease the side effects of long-term opioid use remains unknown.

In summary, due to the vital role of the dopaminergic system on pain modulation, exploiting the multimodal analgesic regimens that target different DA receptors attracts more attention. Thus, we reviewed relevant studies to clarify the potential analgesic features of the DA receptor subtypes, especially D1 and D2 receptors, and summarized as follows: (1) D1 receptors act in different manners with distinct mechanisms in several regions, including the spinal cord, striatum, nucleus accumbens (NAc), and periaqueductal gray (PAG); (2) compared with D1 receptors, D2 receptors may exert a higher analgesic potency. Considering the superadditive or synergistic interactions between the D2 receptors and the opioid receptors, the agonist of D2 receptor may work as an adjuvant to potentiate the analgesic effect and reduce the side effects of opioids [55, 118].

Data Availability

All the researches involved in the present review are available in standard databases such as Web of Science, PubMed, and MEDLINE.

Conflicts of Interest

All authors declare no competing interests.

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Review Article

Bibliometric Study of Pain after Spinal Cord Injury

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Background. The prevalence of comorbid pain after spinal cord injury (SCI) is relatively high in clinical observations and has continued to increase over time. Neuropathic pain (70.14%) is the most popular subject in academic journals after SCI. However, studies that used the bibliometric method to analyze comorbid pain after SCI are still lacking. This study is aimed at combining and integrating acquired information to analyze the global trends of research on the comorbidity of pain after SCI in the last three decades (1990–2019). *Methods*. Systematic works of literature published from 1990 to 2019 were obtained from the Web of Science Core Collection. CiteSpace software was used to analyze the relationship of publication year with the country, institution, journals, authors, references, and keywords. The regression analysis is used to evaluate the percentage of the category increase or decrease over time significantly. IBM SPSS Statistics was used in the statistical analysis. *Results*. A total of 730 publications were included in the analysis. A remarkable increase in the number of publications was observed in the study period ($P < 0.05$). A total of 202 academic journals focused on the categories of clinical neurology, neurosciences, and rehabilitation, and the annual growth rate of articles in these three categories was statistically significant ($P < 0.05$). The USA (356, 48.77%) and the University of Miami (64, 8.77%) were the country and institution with the highest number of publications, respectively. *Spinal Cord*, which was the main journal for research on pain after SCI, had the most publications (88, 12.05%). Burst keywords showed that the individual, inflammation, and central sensitization with pain after SCI are the research development trends and focus in this research field. *Conclusions*. Overall, this study provides the latest research direction for pain after SCI. This historical overview of research into pain after SCI will be a useful basis for further research into development trends, focus issues, cooperators, and cooperative institutions.

1. Introduction

Pain is a frequent complication of spinal cord injury (SCI), and approximately half to two-thirds of patients with SCI suffer from pain; pain after SCI is typically chronic and neuropathic [1–3]. The male-to-female ratio of SCI is high. Most cases are caused by traffic accidents, falls, sport activities, or violence [4]. SCI causes permanent disabilities and brings a heavy burden to people's quality of life, level of functioning, and chances of returning to work [5]. Pain after SCI manifests in many ways. More than 50% of patients with SCI suffered chronic pain within 1 year after SCI [6]. The presence of chronic pain seriously affects the patients' daily life and the social impact. Chronic pain in SCI is related to great emotional distress, and pain hinders the ability of SCI patients to participate in active rehabilitation programs [7]. Acute pain is accompanied by injury and recovery and subsides as tissue scars fade, whereas chronic pain occurs because of poor neuroplasticity [8, 9]. Pain after SCI is difficult to treat because the underlying mechanisms are not yet fully understood. At present, the larger proportion of patients with SCI in most countries and regions is under 30 years old, and the highest incidence rate of 49 every 1 million was recorded in New Zealand [10]. Pain after SCI also brings a medical and economic burden to the government. People with pain after SCI in Canada spend more than US$2.67 billion annually [11].

In view of the high incidence of pain after SCI, a growing number of researchers have studied pain after SCI, and relevant articles have been published in academic journals. A number of randomized controlled trials have studied the treatment of pain after SCI [12–15]. Scientific research

studies increasingly apply bibliometrics in quantitative analysis [16–18]. Bibliometric analysis revealed that research on the application of stem cells in SCI is a rapidly developing research field [19]. However, a quantitative analysis of pain associated with SCI has not yet been conducted.

To address the shortage of quantitative analysis of pain after SCI research, the purpose of this study is to provide a basis for the global scientific research on pain after SCI over the last 30 years (1990–2019). By being able to understand the types of pain after SCI in the past 30 years, current research hotspots provide a theoretical basis for follow-up research. Papers using CiteSpace are on the rise, especially in the medical field, where there have been many related studies [20–22]. CiteSpace 5.6.R5 (Drexel University, Philadelphia, USA) is a commonly used software application for bibliometric analysis. The global research trend on pain after SCI includes four aspects: the number of published papers; the distribution and cooperation between authors, institutions, and countries; a citation burst analysis of keywords; and the cocitation analysis of authors and references.

2. Methods

2.1. Search Strategy. The publications included in this study were those published within the last 30 years (1990–2019). The publications were downloaded from the Science Citation Index Expanded (SCI-Expanded) Web of Science (WoS). The search strategy was as follows: ([TI = spinal cord injury] OR [TI = spinal cord injuries]) AND ([TI = pain] OR [TI = painful]).

2.2. Inclusion Criteria and Exclusion Criteria. Publications related to pain and SCI, such as articles, reviews, letters, and editorial materials, published in different academic journals were included. 872 articles were identified from the Web of Science Core Collection. Conference presentations, meeting abstracts, book reviews, news items, and corrections were excluded. After excluding 136 articles, 736 articles were included. No other specific limitation was imposed except that the chosen language was English. Six non-English language papers were excluded. Finally, 730 articles were included.

2.3. Data Extraction. We followed a previous search strategy to search through the WoS database and then imported the gathered information to CiteSpace for analysis [22–25]. We obtained bibliometric indicators by calculating the number of publications, journals, references, citations, extracted H-index, and keywords. The H-index is a mixed quantitative index that can be used to evaluate the amount and level of the academic output of researchers. The H-index means that an academic journal or researcher has at least H published papers that have at least H citation times per paper. For instance, an H-index of 20 indicates that academic journals or researchers had at least published 20 papers, and the citation frequency is at least 20.

2.4. Statistical Methods. We used CiteSpace 5.6.R5 and Microsoft Excel 2016 to extract and analyze the number of publications (including different journals, countries, institutions, and authors), citation frequency, and keyword trends. We visualized the structure, regular pattern, and distribution of scientific knowledge using CiteSpace and Microsoft Excel:

(1) Analysis of the distribution and trend of journals, countries, institutions, and authors

(2) Analysis of the number of papers, citations, citations per paper, and open-access papers and H-index in the top 10 journals

(3) Assess country-to-country cooperation/institutions/authors

(4) Citation analysis and H-index refer to the number of published papers or research H and at least H paper quality

(5) Analysis of citations and keywords

(6) Cocitation analysis according to references, cited authors, and cited journals

(7) Cooccurrence analysis of terms, keywords, sources, and categories

Besides, we calculated the number of single-author and multiauthor publications, the frequency of WoS subject categories, and types of pain category ranking percentage scores annually (the number of publications every year divided by the total number of publications in each category). The regression analysis is used to evaluate the percentage of the category increase or decrease over time significantly (the category as the dependent variable and the year as the independent variable). SPSS Statistics 22.0 software (Chicago, USA) was used for statistical analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Publication Output and Growth Trends. A total of 730 articles were included (Supplementary Figure 1). According to Figure 1(a), although the number of publications has increased and decreased over the past 30 years, the overall trend has continued to increase. The initial three publications increased to 47 publications from 1990 to 2019 (Figure 1(a)). The number of articles published in 2017 was 66. The results of linear regression analysis indicated that the number of articles published increased significantly with time over the last 30 years ($t = 14.762$, $P < 0.001$). The number of citations increased from 0 citations in 1990 to 2397 citations in 2019. A total of 26,232 citations are cited in all the papers with an average of 874.4 times per year. Figure 1(b) shows that the number of article citations had a significant increase over time ($t = 17.066$, $P < 0.001$). We divided the study period of 30 years into six groups (1990–1994, 1995–1999, 2000–2004, 2005–2009, 2010–2014, and 2015–2019). The largest number of citations per paper was 423.89 from 1995 to 1999. The highest H-index value was 110, and the most cited papers were from 2010 to 2014 (826). The most published papers

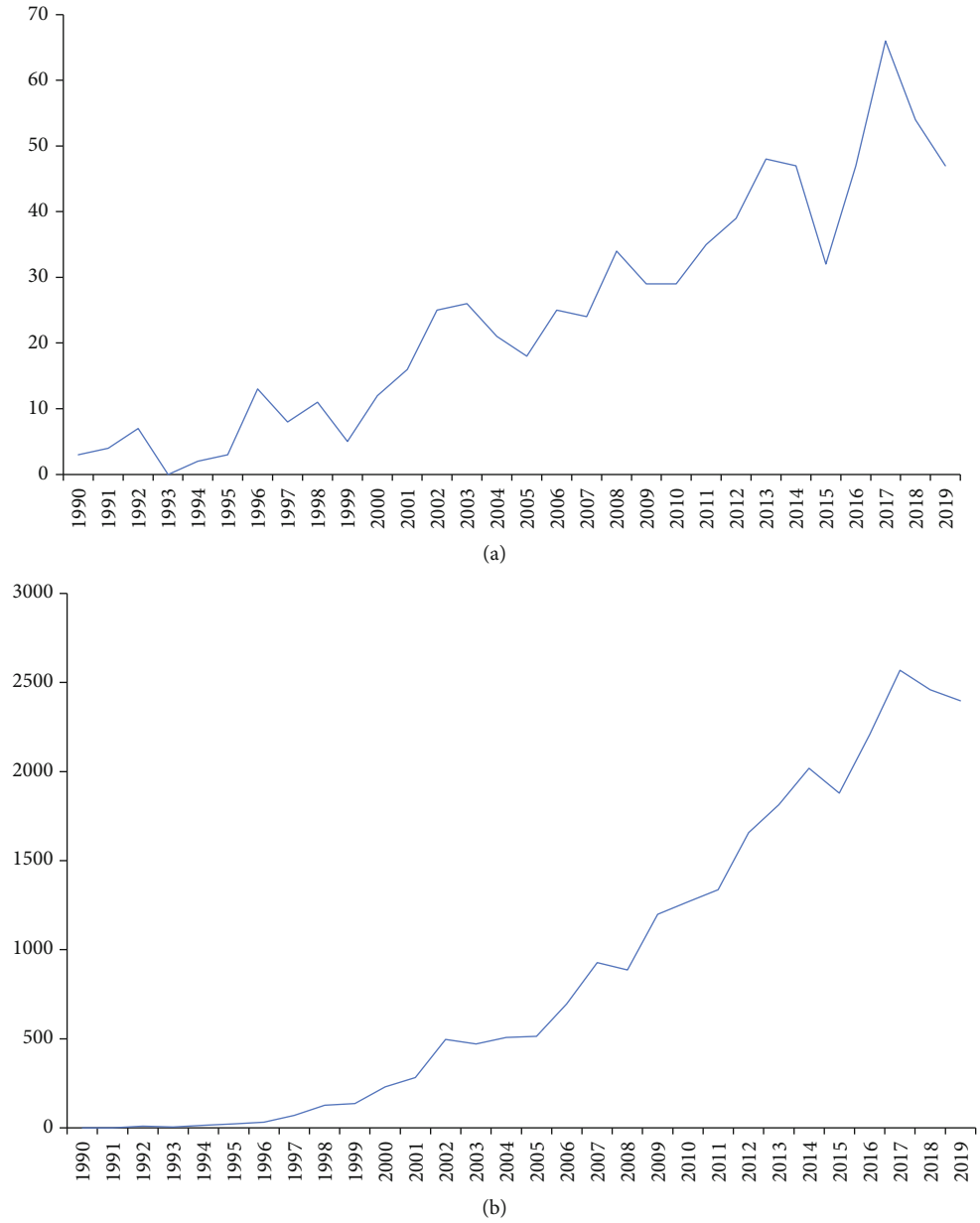


Figure 1: Number of publications and citations. (a) The number of annual publications on pain after spinal cord injury research from 1990 to 2019. (b) The number of annual citations on pain after spinal cord injury research from 1990 to 2019.

(247) and the highest number of open-access papers (145) were recorded in 2015–2019 (Figure 2). And the results of linear regression analysis of the H-index value and the number of open-access papers also have a significant increase with time over the last 30 years ($t = 4.252$, $P < 0.001$; $t = 8.823$, $P < 0.001$).

3.2. Distribution by Journals. Supplementary Table 1 shows that 730 articles were selected through WoS screening, and these 730 articles were published in 202 academic journals. We selected the top 20 of these 202 academic journals according to the number of publications (Table 1). The total number of published articles in the top 20 academic journals exceeded half of the total number of articles (58.91%). The academic journal *Spinal Cord* had published the largest number of articles (88 publications, 12.06%), and its impact factor (IF) is 1.773. *Pain*, which has an IF of 5.483, contributed to the second most published articles (60 publications, 8.22%). The *Archives of Physical Medicine and Rehabilitation* (IF 2019, 3.098; 41 publications, 5.62%), *Journal of Neurotrauma* (IF 2019, 3.793; 33 publications, 4.52%), and *Journal of Spinal Cord Medicine* (IF 2019, 1.816; 28 publications, 3.84%) ranked the third to fifth, respectively, in terms of the number of publications. The *Pain* journal had the highest number of citations (4845) and the highest H-index value (37). *Neurology* had the highest IF amongst the top 20 journals (IF 2019, 8.77), and *Journal of Neuroscience* had the largest number of citations

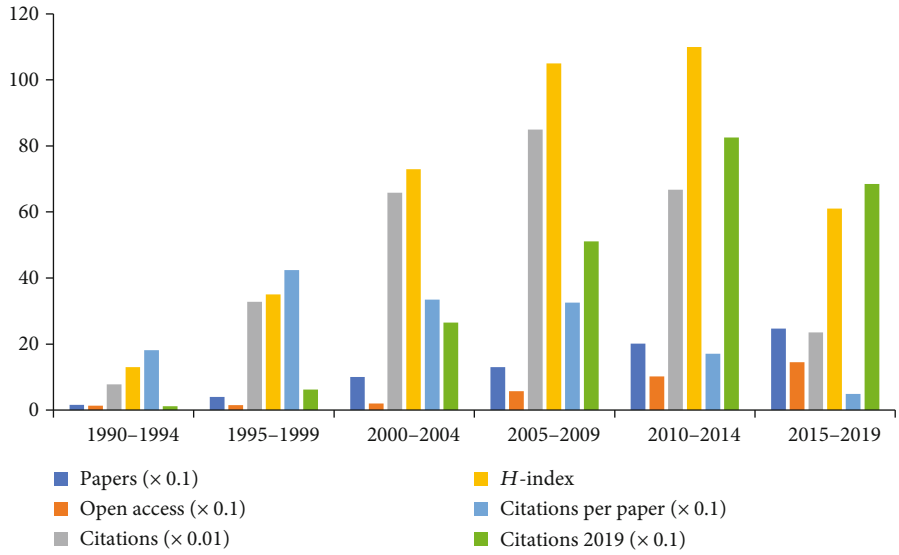


FIGURE 2: Number of papers, citations, citations per paper, open-access papers, and citations in 2019 and H-index for each 5-year time period.

per paper (116.36). In accordance with the journal IF quartile of WoS, 35% of the 20 journals were in the first quartile (Q1), and 45% of the journals were in the second quartile (Q2).

Figure 3 indicates the dual map of the journal. The map on the left represents the citing journals, and the map on the right represents the cited journals. In the dual-map overlay, the labels are marked according to the disciplines of the subject. A line connects the citing journal on the left side to the cited journal on the right side. The dual map indicates that most of the journals were from the molecular, biology, and immunology fields. Simultaneously, most journals were cited from the molecular, biology, and genetics fields.

3.3. Subject Categories of WoS. We classified the 730 articles into the 51 subject categories of WoS and ranked the top 20 journals on the basis of the number of publications (Figure 4). Anesthesiology was the subject category with the largest number of citations per paper (54.85). Clinical neurology had the largest number of publications (355), citations (13,008), and open-access papers (181) and the highest H-index (70).

3.4. Types of Pain. The top 10 types of pain after SCI were ranked as shown in Figure 5. Neuropathic pain (70.14%) is the most popular subject after SCI. Moreover, neuropathic pain had the highest number of publications (512), citations (16,045), and open-access papers (268) and the highest H-index value (77). Notably, average musculoskeletal muscle pain had the most citations per article (67.17).

3.5. Distribution by Countries and Institutions. The 730 articles on pain after SCI were contributed by 42 countries or regions (Supplementary Table 2). Figure 6 shows the top 10 countries or regions according to the number of publications. The United States of America (USA) had the highest number of publications (356), citations (13,874), and open-access papers (168) and the highest H-index value (70), followed by Australia (53), which had the most citations per paper (63.06), and China (53). Figure 7(a) indicates that the contributing countries/regions have extensive and close cooperation and contact. The countries or regions of the included 730 articles are presented in the world map in Figure 8.

A total of 795 institutions (Supplementary Table 3) contributed to 730 papers on pain after SCI. Supplementary Figure 2 indicates the top 10 institutions in terms of the number of published papers. The University of Miami had the largest number of publications (64) and open-access papers (64) and the highest H-index (31). The University of Seattle and the University of Washington had the largest number of citations (2605). The University of Texas Medical Branch Galveston had the largest number of citations per paper (81.56). Figure 7(b) indicates the collaborations between institutions. The University of Miami and the University of Sydney had a strong partnership.

3.6. Distribution by Authors. The 730 articles were contributed by 1000 authors. The top 10 authors and cocited authors were ranked based on the number of journals published (Table 2). Finnerup NB, who published 34 articles, ranked first, followed by Cardenas DD (29 publications) and Siddall PJ (27 publications). Siddall PJ was cocited 403 times, followed by Finnerup NB (269 times) and Widerstrom-noga EG (164 times). Figure 9 indicates the cooperation between authors. Amongst the authors, Cardenas DD not only has many cooperative relations with Jensen MP but also has close cooperation with Turner JA. There is also close cooperation between Finnerup NB, which has the highest number of publications, and Siddall PJ, who ranks third in the number of publications. The proportion of single authors and multiple authors (authors ≥ 2) every 5 years is shown in Figure 10. The linear regression results showed that the percentage of papers with a single author significantly decreased ($t = -3.557$, $P < 0.05$) over time.

Table 1: The top 20 journals of origin of papers in pain after spinal cord injury.

Journals	Papers	Citations (WoS)	Citations per paper	Open-access papers	WoS categories	IF 2019	Quartile	*H*-index
Spinal Cord	88	3164	35.95	84	Clinical neurology; rehabilitation	1.773	Q3; Q2	34
Pain	60	4845	80.75	8	Anesthesiology; clinical neurology; neurosciences	5.483	Q1; Q1; Q1	37
Archives of Physical Medicine and Rehabilitation	41	2109	51.44	5	Rehabilitation; sport sciences	3.098	Q1; Q1	25
Journal of Neurotrauma	33	1127	34.15	12	Clinical neurology; critical care medicine; neurosciences	3.793	Q2; Q2; Q2	19
Journal of Spinal Cord Medicine	28	483	17.25	24	Clinical neurology	1.816	Q3	10
Experimental Neurology	22	1312	59.64	10	Neurosciences	4.691	Q1	17
Clinical Journal of Pain	18	684	38.00	2	Anesthesiology; clinical neurology	2.893	Q2; Q2	14
Journal of Pain	18	684	38.00	7	Clinical neurology; neurosciences	4.621	Q1; Q1	14
Journal of Rehabilitation Research and Development	15	597	39.80	5	Rehabilitation (SSCI); rehabilitation (SCIE)	1.277	Q2; Q3	15
Journal of Neuroscience	14	1629	116.36	12	Neurosciences	5.673	Q1	14
Neuroscience Letters	14	255	18.21	2	Neurosciences	2.274	Q3	10
Disability and Rehabilitation	12	184	15.33	2	Rehabilitation (SSCI); rehabilitation (SCIE)	2.222	Q1; Q1	9
European Journal of Pain	11	225	20.45	4	Anesthesiology; clinical neurology; neurosciences	3.492	Q2; Q2; Q2	6
Journal of Rehabilitation Medicine	11	224	20.36	11	Rehabilitation (SCIE); sport sciences	2.046	Q2; Q2	5
Journal of Pain Research	9	59	6.56	9	Clinical neurology	2.386	Q3	4
Spine	9	440	48.89	1	Clinical neurology; orthopedics	2.646	Q2; Q2	6
American Journal of Physical Medicine Rehabilitation	7	200	28.57	0	Rehabilitation (SCIE); sport sciences	1.838	Q2; Q3	6
Neurology	7	439	62.71	3	Clinical neurology	8.77	Q1	4
PM R	7	100	14.29	2	Rehabilitation (SCIE); sport sciences	1.821	Q2; Q3	4
Molecular Pain	6	152	25.33	6	Neurosciences	2.696	Q3	6

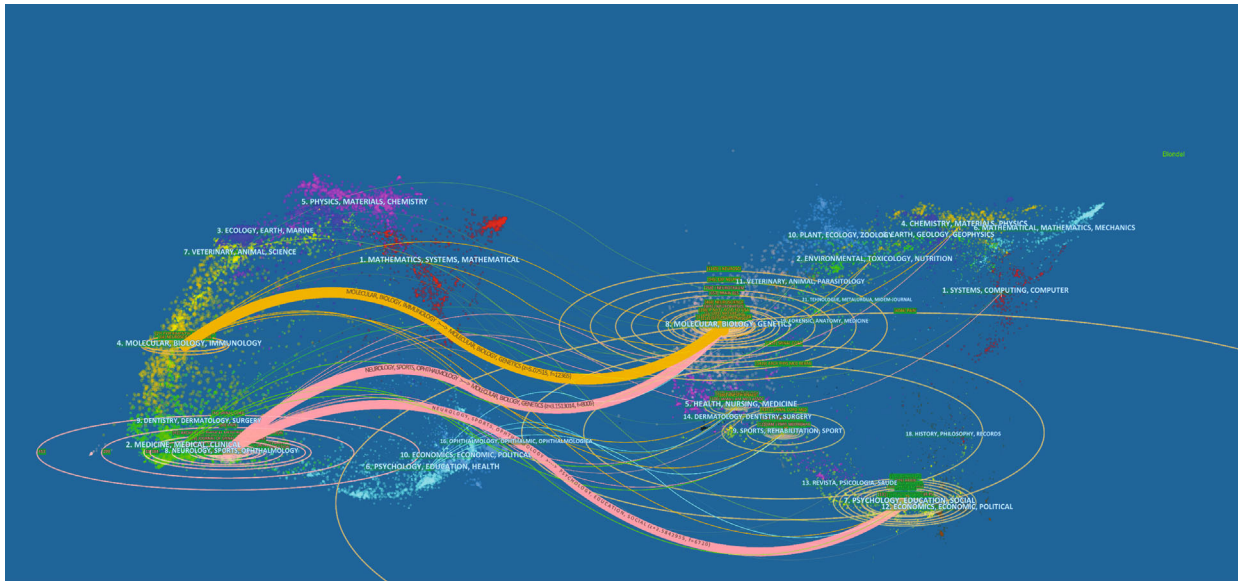


Figure 3: The dual-map overlay of journals related to pain after spinal cord injury.

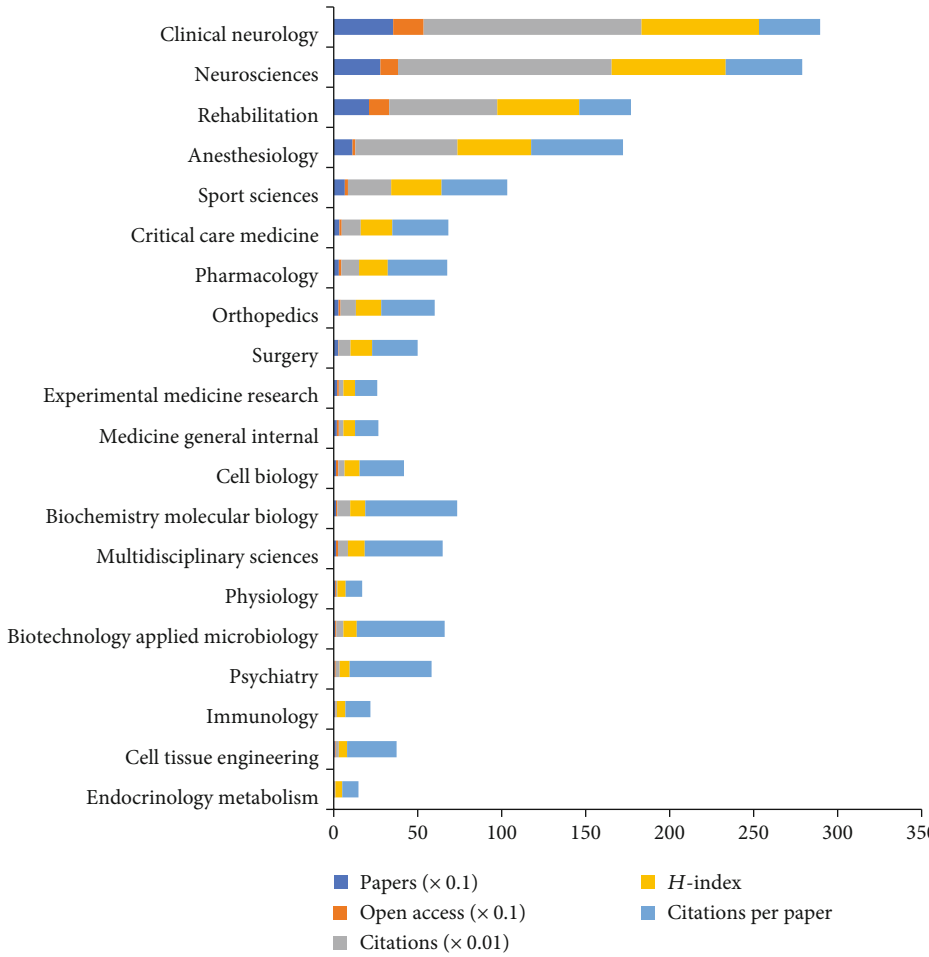


Figure 4: The number of papers, citations, citations per paper, and open-access papers and H-index of the top 20 subject categories of Web of Science.

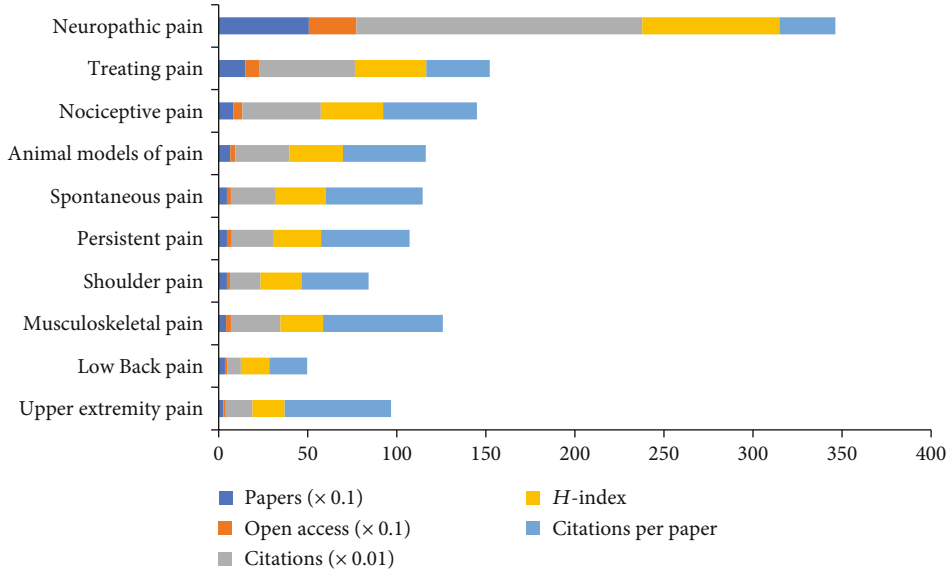


Figure 5: The number of papers, citations, citations per paper, and open-access papers and H-index of the top 10 types of pain.

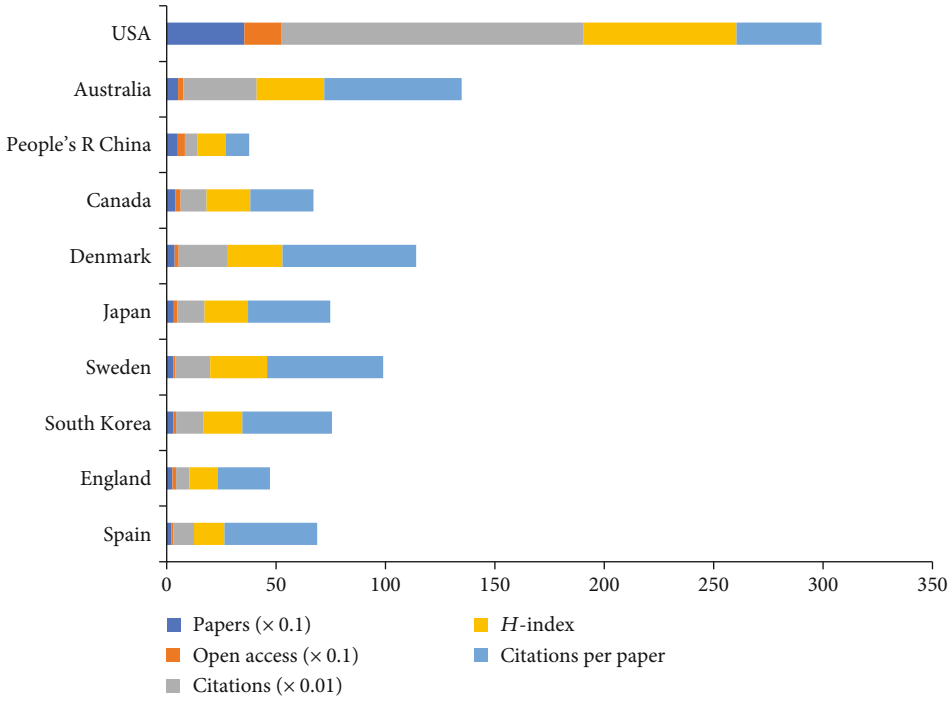
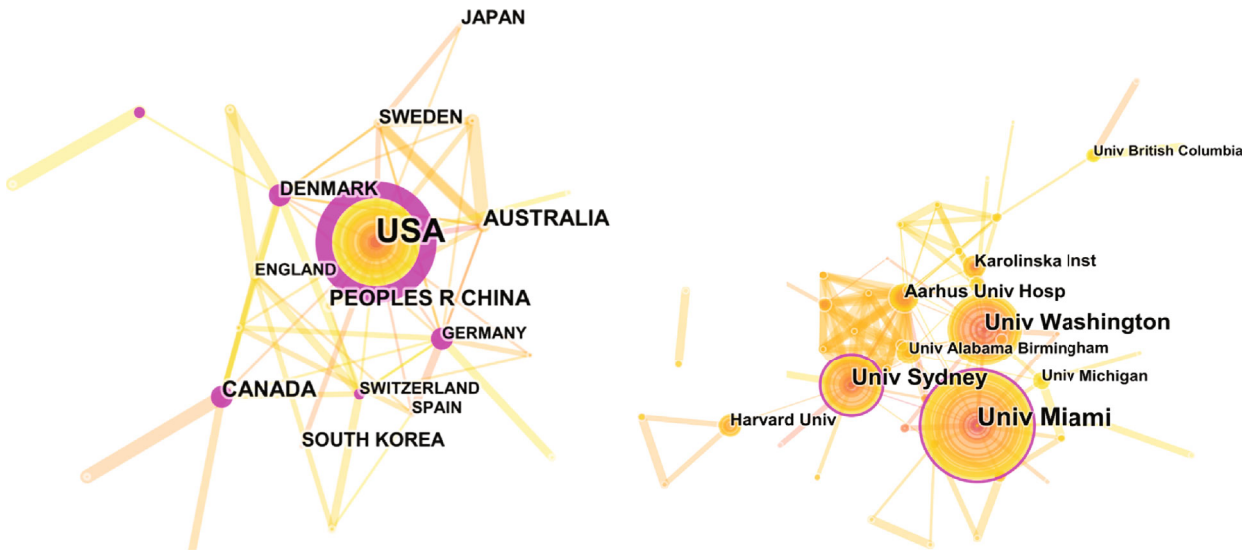


Figure 6: The number of papers, citations, citations per paper, and open-access papers and H-index of the top 10 countries.

3.7. Analysis of References. The cocitation analysis of references is shown in the timeline view in Figure 11. CiteSpace automatically generated the top 17 clusters. The modular Q value shows the significance of the community structure. The modularity Q score was 0.8557 (higher than 0.5), which indicated that the network was reasonably distributed to loosely coupled clusters. The largest cluster was labeled "neurofeedback," the second-largest clusters were "multidimensional" (#1) and "hyperexcitability" (#2), and the third-largest cluster (#3) was "quisqualic acid."

3.8. Analysis of Keywords. The top keyword with the strongest citation burst since 1991 was chronic pain, followed by central pain since 1992 (Figure 12). These keywords with high citation bursts reflect the topic frontier. The current keywords with the strongest citation bursts included



(a) (b)

Figure 7: The analysis of countries and institutions. (a) Network map of countries/territories engaged in pain after spinal cord injury. (b) Network map of institutions engaged in pain after spinal cord injury.

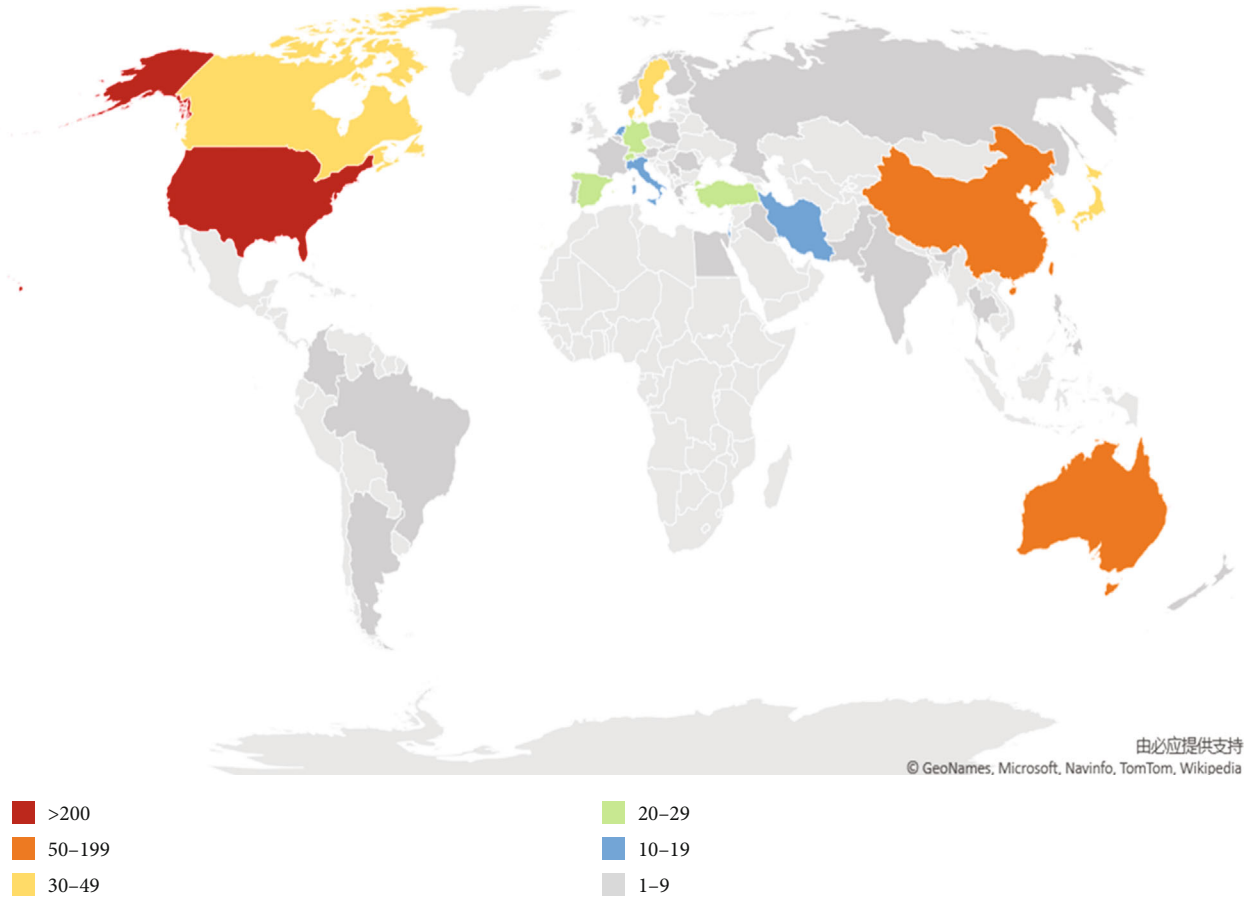


Figure 8: World map of the total country output based on pain after spinal cord injury.

"management" (2015–2019), "quality of life" (2016–2019), "individual" (2016–2019), "inflammation" (2016–2019), and "central sensitization" (2017–2019) amongst the top 26 keywords (chronic pain, central pain, gene-related peptide, lesion, dysesthetic pain, quisqualic pain, pain, questionnaire, double blind, severity, disability, dorsal horn neuron, neuron,

Table 2: The top 10 authors, cocited authors, and cocited references in pain after spinal cord injury.

Author	Published articles	Cocited author	Cited times	Cocited reference	Cited times
Finnerup NB	34	Siddall PJ	403	Siddall PJ, 2003, PAIN, V103, P249, DOI 10.1016/S0304-3959(02)00452-9	67
Cardenas DD	29	Finnerup NB	269	Bryce TN, 2012, SPINAL CORD, V50, P413, DOI 10.1038/sc.2011.156	52
Siddall PJ	27	Widerstrom-noga EG	164	Hains BC, 2006, J NEUROSCI, V26, P4308, DOI 10.1523/JNEUROSCI.0003-06.2006	44
Jensen MP	26	Jensen MP	140	Widerstrom-noga E, 2008, SPINAL CORD, V46, P818, DOI 10.1038/sc.2008.64	44
Richards JS	26	Yezierski RP	132	Hulsebosch CE, 2009, BRAIN RES REV, V60, P202, DOI 10.1016/j.brainresrev.2008.12.010	43
Hulsebosch CE	22	Rintala DH	123	Turner JA, 2001, ARCH PHYS MED REHAB, V82, P501, DOI 10.1053/apmr.2001.21855	42
Widerstrom-noga E	16	Hains BC	117	Siddall PJ, 1999, PAIN, V81, P187, DOI 10.1016/S0304-3959(99)00023-8	42
Widerstrom-noga EG	16	Cardenas DD	107	Siddall PJ, 2009, SPINAL CORD, V47, P352, DOI 10.1038/sc.2008.136	39
Yezierski RP	15	Hulsebosch CE	102	Finnerup NB, 2014, J PAIN, V15, P40, DOI 10.1016/j.jpain.2013.09.008	38
Jensen TS	13	Melzack R	99	Finnerup NB, 2012, CURR PAIN HEADACHE R, V16, P207, DOI 10.1007/s11916-012-0259-x	37

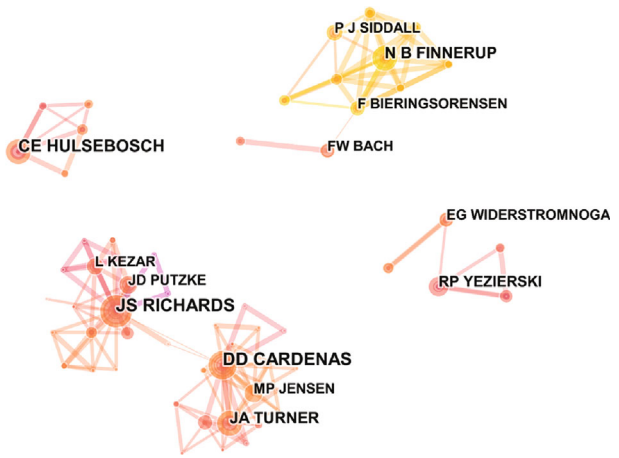


Figure 9: The analysis of authors. Network map of active authors that contributed to pain after spinal cord injury.

tactile allodynia, receptor, hyperexcitability, motor cortex, efficacy, quality, exercise, mechanism, management, quality of life, individual, inflammation, and central sensitization).

3.9. Characteristics of the Top 10 Papers Cited Most Frequently. Table 3 shows the top 10 papers on pain after SCI with the largest number of citations. The 10 papers had 3166 citations, which is 12.07% of all the citations of the included articles. The article of Siddall PJ [26] with the title, "A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury," which was published in 2003 in *Journal of Pain*, was the most cited article (505 citations). The top 10 papers included one [27] journal with IF > 8, five [26, 28–31] journals with 5 ≤ IF < 8, three [32–34] journals with 3 ≤ IF < 5, and one [35] journal with 1 ≤ IF < 3.

4. Discussion

4.1. Global Trends of the Research on the Comorbidity of Pain and SCI. This paper presents a systematic overview by using bibliometric analysis to measure the studies on pain after SCI in the last three decades. The results showed that the global trend of the published works of literature on neuropathic pain after SCI had continued growth over time, indicating that pain after SCI attracted wide attention from researchers and provided a rich foundation for the follow-up research. Although the related publications showed a statistical growth year by year, the fastest growth rate of articles and open-access publications appeared from 2015 to 2019. The fastest growth rate of the number of citations appeared from 2005 to 2009, and the related papers published in 2010–2014 had the highest *H*-index value, indicating that the quality of papers published in the 2005–2014 period was improved.

The top 20 journals contributed to 58.43% (430) of the total number of publications on pain after SCI. *Spinal Cord* had a dominative contribution in terms of the number of works of literature on neuropathic pain and SCI research (11.96%), followed by *Pain* (8.15%), *Archives of Physical Medicine and Rehabilitation* (5.57%), and *Journal of Neurotrauma* (4.484%). The high number of citation frequency and citations per paper implied that *Spinal Cord* and *Pain* had superior quality and academic impression and were known as an unarguable mainstream subject on pain after SCI research. According to Journal Citation Reports (2019 edition), none of the top 20 journals had an IF greater than

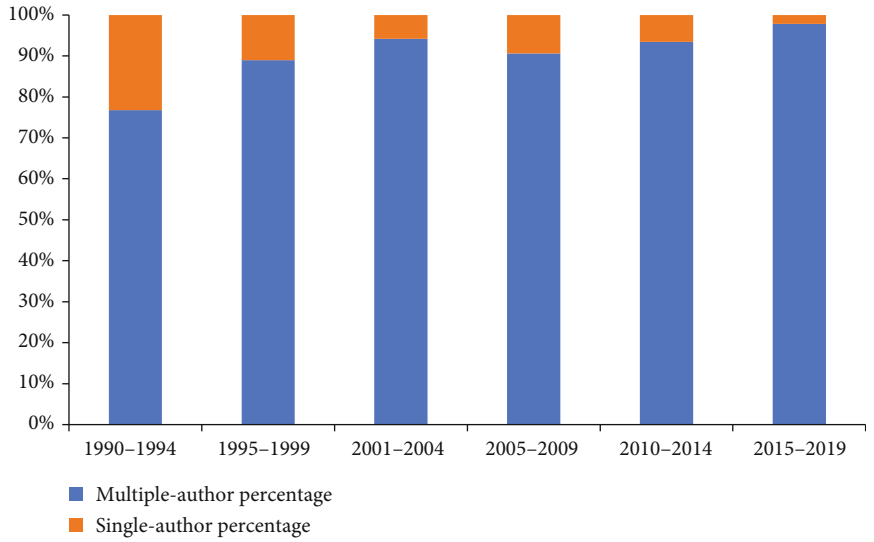


Figure 10: Trends in the percentage of single- vs. multiple-author articles per 5 years.

Figure 11: The analysis of references. Cocitation map (timeline view) of references from publications on pain after spinal cord injury.

10. Seven journals had an IF of 2–3 (*Clinical Journal of Pain*, *Neuroscience Letters*, *Disability and Rehabilitation*, *Journal of Rehabilitation Medicine*, *Journal of Pain Research*, *Spine*, and *Molecular Pain*), four journals had an IF of 3–5 (*Archives of Physical Medicine and Rehabilitation*, *Experimental Neurology*, *Journal of Neurotrauma*, and *European Journal of Pain*), and three papers had an IF of 5–10 (*Neurology*, *Journal of Neuroscience*, and *Neurology*). Amongst the top 20 journals, 35% were in Q1 and 45% were in Q2 according to the journal IF quartile in WoS. There are only three journals with IF > 5, and the average IF of the remaining was 3.265. There was still a challenge in writing in a high IF factor journal.

Based on the quantity of related publications on pain after SCI, the USA had a dominative contribution to the number of works of literature (356), followed by Australia (53), China (52), and Canada (41). The top 10 countries included two American countries, three Asia-Pacific countries, and four European countries. Figure 7(b) shows the expansive network map of the cooperation of the countries by CiteSpace V with 97 nodes and 164 links. The link between the two nodes represents the frequency of cociting articles published by the two nodes, which implies the closeness of the connection between the two nodes. And we can easily acknowledge from Figure 7(b) that the University of Sydney had relatively close collaborations with others. A total of 805 institutions published papers on pain after SCI. Australia had three institutions, and the USA had six institutions (University of Miami, University of Texas System,

Top 26 Keywords with the Strongest Citation Bursts

Keywords	Year	Strength	Begin	End	1990 – 2019
Chronic pain	1990	8.8069	1991	2006	
Central pain	1990	7.2881	1992	2003	
Gene related peptide	1990	3.7558	1996	2000	
Lesion	1990	3.787	1996	2003	
Dysesthetic pain	1990	7.9668	1997	2004	
Paraplegia	1990	3.4511	1997	2002	
Quisqualic acid	1990	3.4511	1998	2003	
Pain	1990	4.5426	1999	2003	
Questionnaire	1990	4.443	2001	2007	
Double blind	1990	6.5092	2002	2004	
Severity	1990	4.1128	2002	2007	
Disability	1990	4.7028	2003	2010	
Dorsal horn neuron	1990	7.6621	2004	2009	
Neuron	1990	5.9142	2008	2010	
Tactile allodynia	1990	3.4543	2008	2012	
Hyperexcitability	1990	3.7249	2011	2013	
Motor cortex	1990	4.1449	2013	2015	
Prevalence	1990	3.8035	2013	2017	
Quality	1990	3.4985	2014	2017	
Exercise	1990	3.5575	2014	2019	
Mechanism	1990	4.1874	2015	2017	
Management	1990	3.6418	2015	2019	
Quality of life	1990	6.7413	2016	2019	
Individual	1990	4.0417	2016	2019	
Inflammation	1990	3.6439	2016	2019	
Central sensitization	1990	3.4558	2017	2019	

Figure 12: The keywords with the strongest citation bursts of publications on pain after spinal cord injury.

University of Sydney, Veterans Health Administration, US Department of Veterans Affairs, and University of Alabama System). These results indicated that the USA was the main power in this field. The top 10 institutions were mainly from the USA with the most publications. The USA, as a developed country, is at the forefront of this research.

4.2. Research Focuses on the Comorbidity of Pain and Spine Core Injury Research. As shown in Figure 4, clinical neurology was the most prolific research field on pain after SCI according to the subject categories of WoS (355), followed by neurosciences (279), rehabilitation (210), and anesthesiology (111). The top 10 subject categories were rehabilitation, clinical neurology, neurosciences, anesthesiology, sport sciences, critical care medicine, pharmacology, orthopedics, surgery, and experimental medicine research. According to the synthetic analysis of the number of publications and citations, the number of citations per paper, and the*H*-index, we could acknowledge that the proportions of the top three subject categories of WoS (clinical neurology, neurosciences, and rehabilitation) were all above 20% and the number of citations per paper was all above 30, implying that the top three subject categories had superior quality and were recognized as the mainstream subject on the pain after SCI research. Based on the types of pain, the majority of the included articles involved neuropathic pain and treating pain after SCI. Amongst the top 10 types of pain (neuropathic pain, treating pain, nociceptive pain, animal models of pain, spontaneous pain, persistent pain, shoulder pain, musculoskeletal pain, low back pain, and upper extremity pain), neuropathic pain had the largest number of publications (506), citations (16,045), and open-access papers (266) and the highest *H*-index value (77), indicating that neuropathic pain has attracted wide attention from researchers, and it is also an urgent problem for patients after spinal cord injury.

Table 3: The top 10 papers with the most citation frequency in pain after spinal cord injury.

Title	First author	Journal	Impact factor	Year	Citations (WoS)	WoS categories	Category ranking
A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury	Siddall PJ	*Pain*	5.483	2003	505	Anesthesiology; clinical neurology; neurosciences	6/32; 25/204; 43/271
A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury	Fregni F	*Pain*	5.483	2006	431	Anesthesiology; clinical neurology; neurosciences	6/32; 25/204; 43/271
Activated microglia contribute to the maintenance of chronic pain after spinal cord injury	Hains BC	*Journal of Neuroscience*	5.673	2006	425	Neurosciences	39/271
Incidence, prevalence, costs, and impact on disability of common conditions requiring rehabilitation in the United States: stroke, spinal cord injury, traumatic brain injury, multiple sclerosis, osteoarthritis, rheumatoid arthritis, limb loss, and back pain	Ma VY	*Archives of Physical Medicine and Rehabilitation*	3.098	2014	353	Rehabilitation in SCIE edition; sport sciences	9/68; 17/85
Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia	Banic B	*Pain*	5.483	2004	305	Anesthesiology; clinical neurology; neurosciences	6/32; 25/204; 43/271
Pregabalin in central neuropathic pain associated with spinal cord injury: a placebo-controlled trial	Siddall PJ	*Neurology*	8.77	2006	261	Clinical neurology	10/204
Upregulation of sodium channel Nav1.3 and functional involvement in neuronal hyperexcitability associated with central neuropathic pain after spinal cord injury	Hains BC	*Journal of Neuroscience*	5.673	2003	253	Neurosciences	39/271
A critical role of toll-like receptor 2 in nerve injury-induced spinal cord glial cell activation and pain hypersensitivity	Kim D	*Journal of Biological Chemistry*	4.238	2007	215	Biochemistry & molecular biology	87/297
Pain following spinal cord injury	Siddall PJ	*Spinal Cord*	1.773	2001	209	Clinical neurology; rehabilitation (SCIE)	150/204; 28/68
Chronic central pain after spinal cord injury	Christensen MD	*Journal of Neurotrauma*	3.793	1997	209	Clinical neurology; critical care medicine; neurosciences	52/204; 10/36; 95/271

In the cocitation map of references, "neurofeedback" was labeled as the largest cluster (#0), the second-largest clusters were "multidimensional pain inventory" (#1) and "hyperexcitability" (#2), and the third was "quisqualic acid" (#3). Based on the analysis of keywords, "chronic pain" had the strongest citation bursts since 1991. The top 26 keywords by the end of 2019 included "exercise" (2014–2019), "management" (2015–2019), "quality of life" (2016–2019), "individual" (2016–2019), "inflammation" (2016–2019), and "central sensitization" (2017–2019). Because of the multiple causes of neuropathic pain, this research area is very broad. However, these publications were mainly focused on neurofeedback and pain management. Patients after spinal cord injury inevitably suffer from different degrees and types of pain, so pain management is becoming more and more important. In addition, emerging interventions to manage pain are diverse, including exercise therapy that is not limited by venue and time. At present, many clinical studies [36–39] have shown that patients with spinal cord injury have good compliance with exercise therapy to relieve pain. Sumizono et al. [36] and Ditor et al. [38] have confirmed aerobic exercise can significantly alleviate neuropathic pain in patients with spinal cord injury. And the finding of Sumizono et al. [36] indicated that aerobic exercise alleviated neuropathic pain through the regulation of glial cell activation and expression of BDNF in the ipsilateral spinal dorsal horn and the endogenous opioid system. In addition, the result of Detloff et al.'s [40] study also suggested that there is a critical therapeutic window when exercise therapy may be effective at treating SCI-induced allodynia and that there are postinjury periods when exercise can be deleterious.

4.3. Strengths and Limitations. This study was the first to combine and integrate acquired information for the bibliometric analyses of the focus issues, direction, and development trend of research studies about pain after SCI over the last 30 years. These publications were retrieved from the SCI-Expanded WoS. The publication selects a variety of journals to ensure the integrity and diversity of the data. Our study included 730 articles on pain after SCI that were published in academic journals, such as *Pain*, *Neurology*, and *Journal of Neuroscience*. Subject categories, the number of publications and citations, the H-index in WoS, the collaborative analyses of journals and countries or institutions, and the cocitation analyses of references or authors and keywords were included.

This study has several limitations. This study only selected the SCI-Expanded WoS database for the retrieval of articles, and non-English papers were not included. Therefore, these factors may cause publication bias. Some influential papers may not be highly cited, whereas others are frequently cited, and the results are widely known.

5. Conclusions

This study provides the latest research direction for pain after SCI. This analysis can enable research teams to collaborate and promote the clinical management of pain after SCI. The initial three publications substantially increased to 47 publications from 1990 to 2019. The USA contributed the greatest number of published articles, and *Neurology* was the most influential journal on pain after SCI. Although this study has some limitations, it showed the common types of pain after SCI, especially neuropathic pain. According to the type of pain, 512 papers focused on neuropathic pain; thus, neuropathic pain is the most common type of pain after SCI. This historical overview of research into pain after SCI will be a useful basis for further research into development trends, focus issues, cooperators, and cooperative institutions.

Data Availability

All research data used to support the findings of this study are included within the article and the supplementary information file.

Conflicts of Interest

The authors report no conflicts of interest.

Authors' Contributions

Yi-Zu Wang and Cheng-Cheng Wu contributed equally to this work.

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Supplementary Materials

Supplementary 1. Supplementary Figure 1: overview of the paper selection process. Supplementary Figure 2: the number of papers, citations, citations per paper, open-access papers, and H-index of the top 10 institutions.

Supplementary 2. Supplement Supplementary Table 1: raw data on journal sources of pain after spinal cord injury. Supplementary Table 2: raw data on countries/territories involved in pain after spinal cord injury. Supplementary Table 3: raw data on institutions involved in pain after spinal cord injury.

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Research Article

Cortical Representations of Transversus Abdominis and Multifidus Muscles Were Discrete in Patients with Chronic Low Back Pain: Evidence Elicited by TMS

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Introduction. The transversus abdominis (TVA) and multifidus (MF) muscles are the main segmental spinal stabilizers that are controlled by the primary motor cortex of the brain. However, relocations of the muscle representation in the motor cortex may occur after chronic lower back pain (cLBP); it still needs more evidence to be proven. The current study was aimed at applying transcranial magnetic stimulation (TMS) to investigate the changes of representation of TVA and MF muscles at the cortical network in individuals with cLBP. *Methods*. Twenty-four patients with cLBP and 12 age-matched healthy individuals were recruited. Responses of TVA and MF to TMS during muscle contraction were monitored and mapped over the contralateral cortex using a standardized grid cap. Maps of the center of gravity (CoG), area, volume, and latency were analyzed, and the asymmetry index was also computed and compared. *Results*. The locations of MF CoG in cLBP individuals were posterior and lateral to the CoG locations in healthy individuals. In the healthy group, the locations of TVA and MF CoG were closed to each other in both the left and right hemispheres. In the cLBP group, these two locations were next to each other in the right hemisphere but discrete in the left hemisphere. In the cLBP group, the cortical motor map of TVA and MF were mutually symmetric in five out of eleven (45.5%) subjects and leftward asymmetric in four out of ten (40.0%) subjects. *Conclusions*. Neural representations of TVA and MF muscles were closely organized in both the right and left motor cortices in the healthy group but were discretely organized in the left motor cortex in the cLBP group. This provides strong support for the neural basis of pathokinesiology and clinical treatment of cLBP.

1. Introduction

Low back pain (LBP) affects people of all ages and is the third major contributor to the global burden of disability [1]. Pain in the lumbar area with or without accompanying buttock pain over 12 weeks was defined as chronic LBP (cLBP) [2, 3]. The etiology of LBP is complex and involves multiple systems which requires further evidence to clarify the efficacy on interventions for this pathological condition [1]. The evidence that supports structural and functional changes within the central nervous system of people with cLBP is increasing, which appears to play a prominent role in the pathophysiology of these disorders [4, 5]. These neuroplastic changes are reflective of adaptive neurophysiological processes occurring as the result of altered afferent stimuli and cortical areas with cLBP that are initially beneficial, but may persist in a chronic state, and may be part and parcel in the pathophysiology of the condition and the development and maintenance of chronic signs and symptoms [5, 6].

Previous studies suggested that the transversus abdominis (TVA) and multifidus (MF) muscles are the primary segmental spinal stabilization muscles [7] that are controlled

by the primary motor cortex of the brain [8, 9]. Understanding how these muscles cooperate to influence lumbopelvic stability is critical to anatomical and biomechanical analysis, as well as to the implementation of effective treatment in patients with LBP. To date, little is known about the relationship between TVA and MF muscles in the cortical representation, which are the local abdominal and back muscles to stabilize the lumbar spine [7]. An anatomical study of the relationship between TVA and MF muscles showed a codependent mechanism that involved a balanced tension between deep abdominal and lumbar spinal muscles, which are linked through the aponeurotic components of the thoracolumbar fascia [10]. A point of equal tension may exist between the MF and TVA muscles [10]. The ability to contract MF was also related to the ability to contract TVA [11], and a poor MF contraction was related to a poor TVA contraction. However, a cross-sectional study found that it was the MF muscle activation, rather than the TVA muscle, that is associated with successful clinical decision using stabilization exercises for patients with cLBP [12]. In addition, some research revealed that individuals with cLBP had increased fatigue of the MF, decreased activation of the TVA [13], and reduced MF muscle thickness at rest and during contraction as compared to healthy individuals [14].

Transcranial magnetic stimulation (TMS) has been used for decades primarily to evaluate changes in the motor cortex in the presence of neurophysiological diseases and to assess therapeutic effects after stimulation [15, 16]. As a noninvasive technique, TMS enables the investigation of the relationship between disorders in the musculoskeletal system and functional changes in the brain, including the adaptive changes of the motor cortex related to the TVA or MF muscles in patients with LBP [2, 3, 17, 18]. The center of gravity (CoG) is known as a robust measure of motor cortical representation and corresponds closely to the area of high excitability of corticomotor neurons that project to the target muscle(s) [19]. A study found that the CoG of the motor cortical mapping of TVA was approximately 2 cm anterior and lateral to the vertex in the healthy group, but the CoG in the LBP group was more posterior and lateral to the CoG location in the healthy group [2]. Another result among healthy people showed that the motor cortical representation for MF was located posterior to that for the erector spinae [3]. In patients with LBP, the short-interval intracortical inhibition level was lower in the left hemisphere and MF volitional contraction was not related to motor cortex excitability [17]. These findings provided preliminary evidence of the reorganization of deep abdominal and lumbar spinal muscle representation in the motor cortex [3, 17, 18], which indicates that using TMS to evaluate the relationship between cortical reorganization and changes in trunk stabilizing muscles does offer a unique insight into building links between the brain (control subsystem), muscles (active musculoskeletal subsystem), and LBP (pain).

Previous studies of TVA and MF muscles' function in the presence of cLBP focused mostly on electromyography (EMG) signals and muscle size in isolation [13, 14], and the studies of the relationship between the two muscles have been limited to the clinical qualitative outcome. Therefore, the first purpose of this study was to compare the cortical motor representation of the TVA and MF muscles in healthy individuals and cLBP patients. The second purpose of this study was to find the relatively changed relationship between TVA and MF in the motor cortex in individuals with and without cLBP. We hypothesized that the CoGs of the cortical motor representation of the two muscles were different between patients with cLBP and healthy individuals and that patients with low back pain have a more discrete relation between the two muscles when compared with healthy individuals. TMS is capable in identifying changes in cortical motor representation; thus, the study protocol was adequately designed to investigate the relationship between the two muscles at the cortical level.

2. Materials and Methods

2.1. Subjects. Subjects were recruited from the local rehabilitation ward and outpatient department of the hospital. The inclusion criteria for cLBP subjects were as follows: (1) pain in the lumbar area with or without accompanying buttock pain over the past three months [2], (2) pain intensity (perceived during the week preceding the experiment and at the end of experiment) assessed by the 0–10 Numerical Pain Rating Scale (NPRS) with a score ranging from 3 to 7 [20], and (3) ability to perform the experiment procedure. Exclusion criteria were (1) the active existence of respiratory, orthopaedic, circulatory, nephrological, or neurological dysfunctions; (2) previous surgery to the abdomen or lower back; (3) female subjects who were pregnant or suffered from dysmenorrhea; and (4) epilepsy or a family history of epilepsy.

The study protocol was approved by the Medical Ethical Committee of the First Affiliated Hospital, Sun Yat-sen University ([2017]250), and all subjects provided written informed consent before the experiment. The study was conducted in accordance to the Declaration of Helsinki.

2.2. Transcranial Magnetic Stimulation. A 7 cm figure-of-eight coil, connected to a Rui Chi magnetic stimulator (Yiruide CCY-IA, Wuhan, China) with a maximum stimulator output (MSO) of 2.0 T, was used to map the neuronal networks of the motor cortex associated with excitation of the contralateral TVA and MF muscles. The figure-of-eight coil provides a better focality of stimulation compared to the standard circular coil and is more ideal for mapping of the motor cortex, as it would evoke fewer ipsilateral responses [2]. The stimulating coil was placed in a crossover position over standardized scalp grids, with the coil handle pointing backward and laterally 45° away from the anterior-posterior axis [2, 3].

2.3. EMG Recordings. EMG responses of TVA and MF muscles were detected with two surface electrodes (Ag/AgCl discs, interelectrode distance 2.0 cm, Noraxon, USA). In the healthy control group, bilateral TVA and MF muscles were selected. In the cLBP group, the TVA and MF of the more painful side were selected. The locations of the EMG electrodes were determined in accordance with EMG placement guidelines [21] and published studies [2, 17, 22]. All electrodes were connected to an EMG recording system (Yiruide, Wuhan, China). The motor-evoked potential (MEP) recordings were digitized

with a sample rate of 100 kHz, amplified and filtered with a bandpass of 2~10 kHz and a noise eliminator of 50 Hz, and then stored for offline analysis.

2.4. Pressure Biofeedback Unit. The Pressure Biofeedback Unit (PBU) (Chattanooga Group Inc., LLC, Vista, California, USA) employed in this study is a widely used noninvasive device for the monitoring of the contraction status of the TVA and MF muscles in patients with LBP in a seated position [22]. Our previous study showed that when the pressure reached 50 mmHg in the seated position, the target for the voluntary contraction of MF and TVA could reach 11.55% MVC (maximum voluntary contraction) and 13.75% MVC, respectively [22]. The pressure of 50 mmHg was a comfortable level for subjects to maintain and minimized the potential of fatigue. The target pressure was displayed on a monitor and the real-time feedback of the pressure was shown to the subject.

2.5. TMS Testing of MF and TVA Muscles. A grid cap was precreated on the sculpture head model [2]. It was a 6×7 cm grid system over each hemisphere, from the midline to 6 cm lateral to the vertex and from 2 cm posterior to 5 cm anterior of the vertex [2, 17]. Each standard 1×1 cm scalp grid box was numbered to record data and make topographic maps (Figure 1). The inter- and intraexaminer validity of the grid cap mapping system was reported to be excellent [23].

Subjects sat comfortably upright against the chair with their arms well supported on their legs and both feet resting flat on the floor. A tight-fitting standardized scalp elastic cap was worn over the head [24]. The skin was cleaned, and the electrodes were placed at the position in accordance with published studies [2, 17, 22]. The ground pole was connected at the ipsilateral wrist of the measured muscle. To locate the optimal stimulus site for the left or right TVA or MF, the contralateral motor cortex around the anatomical cortex area was stimulated from a single pulse with 70% of the maximum stimulator output (MOS) [2, 25]. The site at which TMS consistently elicited the largest MEPs was determined as the TVA or MF "hot spot." We took 70% MOS as the baseline, then increased or decreased by 5% MOS increments until an intensity was found that evoked reliable MEPs ($\geq 50\,\mu$V in amplitude) in at least five of 10 consecutive trials. The lowest stimulus intensity was determined as the resting motor threshold (RMT) [2, 25].

After RMT was determined, the coil intensity was set to 120% RMT. Subjects were asked to push the pressure cell up to 50 mmHg. Random stimulation was delivered over each point on the cap grid in order to avoid selective bias. Ten stimuli were delivered at each 1×1 cm grid at a pressure of 50 mmHg with an interstimulus interval of at least 5 s [2, 3, 17]. We stimulated the contralateral motor cortex of the target muscle. The order of hemispheric stimulation (right or left) was randomized with an equal number of stimulation sequences within the healthy group. An interval of 48 hours was placed between the stimulation of each hemisphere to avoid the interaction effect of TMS on either hemisphere. In patients with cLBP, the contralateral motor cortex of the more painful side was examined. EMG responses from each grid and muscle group were recorded. A point was marked positive when at least five of 10 stimulations evoked reliable MEP ($\geq 50\,\mu$V in amplitude). Grid points were stimulated outward from the center until a positive area was demarcated by negative points (MEP $< 50\,\mu$V). MEP data were stored for offline analysis. This study uses the method of Tsao et al. and the method description partly reproduces their wording [2, 3].

2.6. Data Analysis. MEP amplitude was defined as the peak-to-peak voltage of the EMG responses. Five TVA and MF MEPs were averaged at each scalp site. A topographical map of the amplitude of the responses of each muscle was produced by superimposing the MEPs over the respective scalp sites. All responses were normalized to the amplitude of the peak response. Normalized values below 25% of the peak response were removed [2, 17].

Three parameters of the map were calculated from the normalized maps. Map volume, a measure of the total excitability of cortical representation, was calculated as the sum of normalized MEP amplitudes recorded at all scalp sites where responses were evoked. CoG location of the map was calculated using the formula Equation (1), where x_i and y_i are medial-lateral and anterior-posterior locations and z_i is the normalized amplitude [2, 3, 17]. This measure gives an amplitude weighted indication of the map position [2, 3, 17]. The map area was identified as the sites on the scalp grid over each hemisphere from which an EMG response was obtained.

$$\mathrm{CoG} = \frac{\Sigma z_i x_i}{\Sigma z_i}, \frac{\Sigma z_i y_i}{\Sigma z_i}. \tag{1}$$

Latency was defined as the interval from stimulus onset to the individual muscle EMG response. The mean value of the five shortest latencies for each muscle was calculated for analysis [26]. The asymmetry index (AI) was calculated according to the formula Equation (2); L and R represent the map area covering the left and right hemispheres, respectively [27]. Functional hemispheric lateralization was defined as follows: $\mathrm{AI} > 0.10$ as left-sided lateralization, $\mathrm{AI} < -0.10$ as right-sided lateralization, and $-0.10 \leq \mathrm{AI} \leq 0.10$ as mutual lateralization [27]. Python 3.7 (Spyder) was used to create the 3-D plots of the representative maps.

$$\mathrm{AI} = \frac{L - R}{L + R}. \tag{2}$$

2.7. Statistical Analysis. Statistical analysis was conducted using the SPSS 25.0 software (SPSS Inc., Chicago, IL, USA). Values of dependent variables in each group were described in mean and standard deviations. The Kolmogorov-Smirnov test was used to test the distribution normality of the data. All the variables were normally distributed ($P > 0.05$). Then, a two-way ANOVA was performed to compare the effects of Group (healthy and cLBP) and Side (left and right) of each muscle to assess map volume, area, CoG location (medial-lateral locations and anterior-posterior locations), and latency. If the main effect of the groups was significant, an independent sample t-test was conducted to compare the differences between two groups or between two sides. A significant level was set at $P < 0.05$.

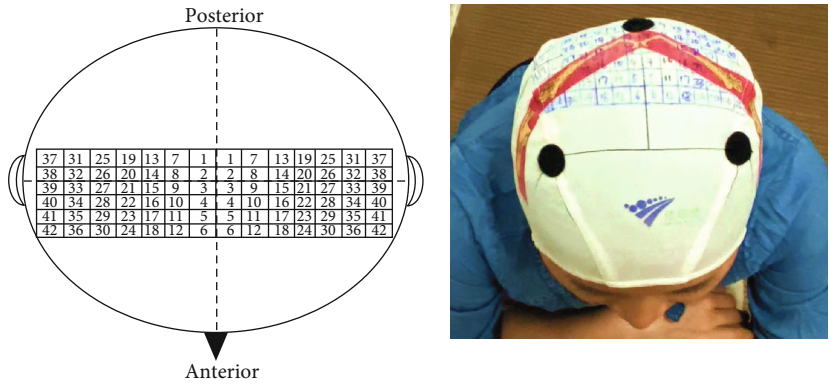

Figure 1: The standard 1 × 1 cm grid cap. (a) Schematic diagram. L: 6 × 7 cm grid; R: 6 × 7 cm grid. (b) Rendering figure. The red band is the primary motor cortex. The two black dots on the ventral side are the left and right dorsolateral frontal lobes. The black dot on the dorsal side is at 1 cm behind the central zero point.

3. Results

Twenty-four right-handed individuals with cLBP and 12 right-handed healthy individuals with no history of LBP were recruited (Table 1). There was no group difference in gender, age, height, weight, BMI, and educational level ($P > 0.05$). TVA's MEPs could not be evoked over one hemisphere in one healthy subject and one subject with cLBP. The MEPs of MF could not be evoked over one hemisphere in one healthy subject and two subjects with cLBP when using a stimulation intensity of MSO (RMT > MSO).

3.1. TMS Mapping. Figure 2 shows the average normalized motor cortical representation maps of TVA and MF responses to TMS for the healthy and cLBP groups in the left and right hemispheres. Both the locations of TVA and MF CoG for cLBP were located posterior and lateral to the CoG location in healthy individuals in the left and right hemispheres. For the TVA muscle, there was no statistically significant Group ∗ Side interaction effect (map area: $F_{\text{interaction}}(1, 38) = 0.965$, $P = 0.332$; map volume: $F_{\text{interaction}}(1, 38) = 0.401$, $P = 0.530$; latency: $F_{\text{interaction}}(1, 38) = 0.147$, $P = 0.704$), the main effect of Group was not significant (map area: $F_{\text{group}}(1, 38) = 0.023$, $P = 0.880$; map volume: $F_{\text{group}}(1, 38) = 0.017$, $P = 0.896$; latency: $F_{\text{group}}(1, 38) = 0.008$, $P = 0.929$), and the main effect of Side was also not significant (map area: $F_{\text{side}}(1, 38) = 0.634$, $P = 0.431$; map volume: $F_{\text{side}}(1, 38) = 0.678$, $P = 0.415$; latency: $F_{\text{side}}(1, 38) = 0.005$, $P = 0.945$). Similar results were found in the MF muscle in that no significant interaction effect or main effects were revealed (map area: $F_{\text{interaction}}(1, 34) = 0.497$, $P = 0.485$; $F_{\text{group}}(1, 34) = 1.811$, $P = 0.187$; $F_{\text{side}}(1, 34) = 1.099$, $P = 0.302$; map volume: $F_{\text{interaction}}(1, 34) = 0.218$, $P = 0.644$; $F_{\text{group}}(1, 34) = 2.457$, $P = 0.126$; $F_{\text{side}}(1, 34) = 1.758$, $P = 0.194$; latency: $F_{\text{interaction}}(1, 34) = 0.498$, $P = 0.485$; $F_{\text{group}}(1, 34) = 0.017$, $P = 0.896$; $F_{\text{side}}(1, 34) = 0.171$, $P = 0.682$) (Figures 3(a)–3(c)).

3.2. The Relationship between TVA and MF in the Motor Cortex. Figure 3(d) shows the relationship between TVA and MF in the left and right hemispheres of the healthy and cLBP groups. In the healthy group, the CoGs of the TVA and MF muscles are closed to each other in both the left and right hemispheres. In the cLBP group, they are closed at the right hemisphere, whereas at the left hemisphere, they are obviously discrete. For the TVA muscle, there was no statistically significant Group ∗ Side interaction effect (maps CoG of the medial-lateral coordinates: $F_{\text{interaction}}(1, 38) = 0.020$, $P = 0.889$; maps CoG of the anterior-posterior coordinates: $F_{\text{interaction}}(1, 38) = 0.900$, $P = 0.349$) and the main effect of Side was not significant either (maps of CoG of the medial-lateral coordinates: $F_{\text{side}}(1, 38) = 1.497$, $P = 0.229$; maps of CoG of the anterior-posterior coordinates: $F_{\text{side}}(1, 38) = 0.732$, $P = 0.397$). It is worth noting that a significant main effect of Group was found (maps of CoG of the medial-lateral coordinates: $F_{\text{group}}(1, 38) = 12.267$, $P = 0.001$; maps of CoG of the anterior-posterior coordinates: $F_{\text{group}}(1, 38) = 16.121$, $P < 0.0001$).

For the MF muscle, the results of two-way ANOVA tests indicated no statistically significant Group ∗ Side interaction effect (maps of CoG of the medial-lateral coordinates: $F_{\text{interaction}}(1, 34) = 2.740$, $P = 0.107$; maps of CoG of the anterior-posterior coordinates: $F_{\text{interaction}}(1, 34) = 0.026$, $P = 0.872$). A significant main effect of Side was found (maps of CoG of the medial-lateral coordinates: $F_{\text{side}}(1, 34) = 4.846$, $P = 0.035$; maps of CoG of the anterior-posterior coordinates: $F_{\text{side}}(1, 34) = 4.762$, $P = 0.036$). Again, a significant main effect of Group was found (maps of CoG of the medial-lateral coordinates: $F_{\text{group}}(1, 34) = 9.340$, $P = 0.004$; maps of CoG of the anterior-posterior coordinates: $F_{\text{group}}(1, 34) = 25.019$, $P < 0.0001$). Both of the TVA and MF maps of CoG of the medial-lateral and anterior-posterior coordinates show a significant difference in the healthy and cLBP groups ($P < 0.05$) except the MF on the right side of the medial-lateral locations ($P = 0.420$) (Table 2).

3.3. Interhemispheric Asymmetry of TVA and MF Muscles. Interhemispheric asymmetry was found in the two groups (Table 3). In the healthy group, both the cortical motor maps of TVA and MF were leftward asymmetric in five out of

Table 1: Characteristics of the sample cohorts (mean ± (SD)).

Demographics	cLBP ($n = 24$)	Healthy ($n = 12$)	P value
Gender (M : F)	11 : 13	6 : 6	—
Age (years)	28.75 (4.13)	28.17 (4.00)	0.69
Height (cm)	167.92 (9.02)	167.83 (8.08)	0.98
Weight (kg)	61.13 (11.13)	60.75 (9.12)	0.92
BMI (kg/m^2)	21.48 (2.03)	21.44 (1.89)	0.89
Education level (years)	18.96 (2.63)	20.08 (2.83)	0.27
Side of pain (L : R)	12 : 12	—	—
Pain intensity (NPRS)	4.63 (1.13)	—	—
Pain duration (years)	2.44 (1.72)	—	—
ODI (%)	20.29 (9.32)	—	—

cLBP: chronic low back pain; BMI: body mass index; ODI: Oswestry Disability Index; L: left; R: right; NPRS: numerical pain rating scale; SD: standard deviation.

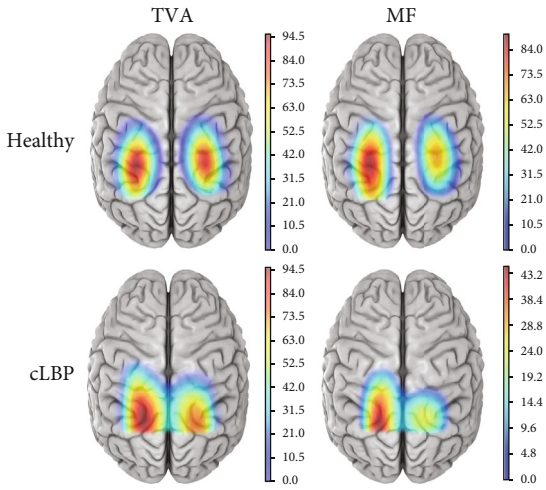

Figure 2: Average representative location of TVA and MF responses to TMS for the healthy and cLBP groups on the left and right hemispheres. cLBP: chronic low back pain; L: left; R: right; TVA: transversus abdominis; MF: multifidus; CoG: center of gravity. Note: this figure was generated from the average data of all the patients. The posterior aspect of the map appeared to be not recorded fully over the entire cortical representation of the muscle. This was due to two cortical motor maps of cLBP patients located more posteriorly. The CoG of TVA and MF from all other participants' muscles in cLBP patients were within the region of the grid cap.

eleven (45.5%) subjects. In the cLBP group, the cortical motor map of TVA was mutually symmetric in five out of eleven (45.5%) subjects while the cortical motor map of MF was leftward asymmetric in four out of ten (40.0%) subjects.

4. Discussion

This study applied TMS to investigate the changes of representation of TVA and MF muscles at the cortical network in individuals with and without cLBP. The results showed discrete organization for the representation of TVA and MF muscles in the left motor cortex and are mutually symmetrical in the cLBP group. Meanwhile, the representation of TVA and MF muscles is closely organized in the right and left motor cortices and is leftward asymmetric in the healthy group. We also found that both the locations of TVA and MF CoGs for cLBP were located posteriorly and laterally compared to those CoG locations observed in healthy individuals. These novel findings revealed the differences between TVA and MF muscle representations in the motor cortex between healthy and cLBP groups.

4.1. TVA and MF Muscles Reorganized in the Motor Cortex in Subjects with cLBP. Our results showed that in healthy individuals, TVA representation was located at 1.91 (0.48) cm anteroposterior in the right hemisphere and 1.63 (0.40) cm anteroposterior in the left hemisphere (Figure 2). However, in cLBP individuals, TVA representation was located at 1.14 (0.63) cm anteroposterior in the right hemisphere and 1.15 (0.47) cm anteroposterior in the left hemisphere (Figure 2). The results showed that the motor cortical map of TVA in the LBP group was more posterior and lateral than that of the healthy group, which was supported by the findings of Tsao et al. [2]. Another study by Tsao et al. [3] reported that MF representation was located at 2.6 (0.3) cm mediolateral and 1.4 (0.4) cm anterior to the vertex in healthy individuals, but MF representation has not been studied in individuals with cLBP. However, our results found that the locations of MF CoG were posterior and lateral in individuals with cLBP compared to healthy individuals at the left and right hemispheres. Moreover, the shift was consistently observed in most individuals with cLBP. Our study, together with other early literatures [2, 3], suggested that the present findings were unlikely to be related to cap displacement or inaccurate identification of the vertex.

4.2. The Relationship between TVA and MF in the Motor Cortex. Our study revealed that the representation between TVA and MF muscles in the left motor cortex was discrete in subjects with cLBP and close to each other in healthy people (Figure 3(d)). Structural relationships from TMS maps could imply changes in the structural or functional organization of cortical networks that are associated with the activation of TVA and MF muscles in the motor cortex [28]. Evidence of close organization between TVA and MF muscles adds weight to the notion that these muscles may play distinct roles in the control of spinal posture and movement in healthy people [2, 17]. With all of these results, it is reasonable to speculate that the cocontraction of TVA and MF muscles maintains lumbar stability, and this cocontraction diminished in people with cLBP [11, 18]. Interestingly, the present study did not find discrete organization for the representation of TVA and MF muscles at the right hemisphere in the cLBP group. It might be related to the fact that all subjects were right-handed. van den Berg et al. [29] tested right-handers and left-handers with TMS, and the results showed that for right-handed participants, more disruptions were induced when TMS was applied over the left M1 region. Other studies also reported that the left hemisphere was associated with the construction and storage of motor programs, monitoring and modification of movements, and selection and retrieval of motor programs for sequential movements [30–32]. Therefore, we inferred that

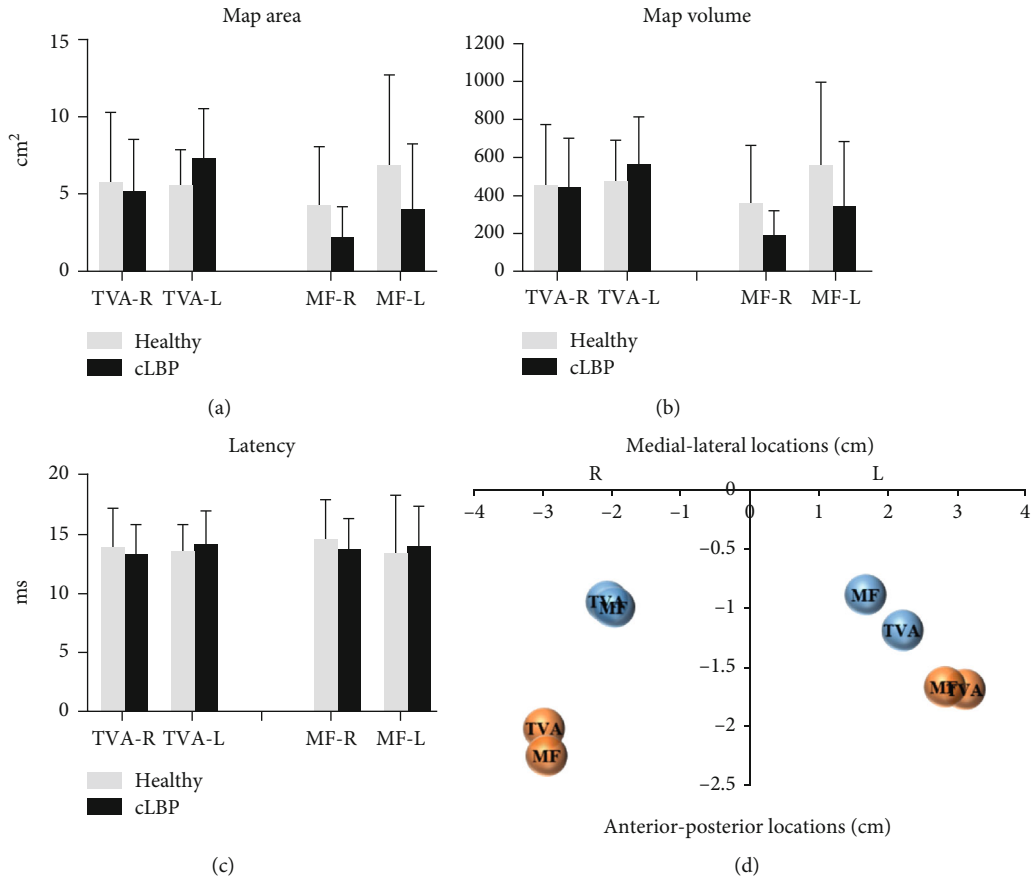

Figure 3: (a–c) Map area, map volume, and latency of TVA and MF MEP responses to TMS for the healthy and cLBP groups on the left and right hemispheres. (d) The relationship between TVA and MF in the left and right hemispheres of healthy and cLBP groups. The blue bubbles denote the CoGs of the muscles in the cLBP group, and the orange bubbles denote the CoGs of the muscles in the healthy group. cLBP: chronic low back pain; L: left; R: right; TVA: transversus abdominis; MF: multifidus; CoG: center of gravity.

Table 2: The CoG of the medial-lateral (x-CoG) and anterior-posterior coordinates (y-CoG) in the left and right hemispheres of healthy and cLBP groups (mean ± (SD)).

	cLBP	Healthy	*P* value
Medial-lateral locations (cm)			
TVA-R	2.14 (0.56)	2.76 (0.44)	0.011
TVA-L	2.34 (0.84)	3.01 (0.45)	0.035
MF-R	2.64 (0.97)	2.92 (0.30)	0.420
MF-L	1.88 (0.45)	2.81 (0.47)	<0.0001
Anterior-posterior locations (cm)			
TVA-R	1.14 (0.63)	1.91 (0.48)	0.005
TVA-L	1.15 (0.47)	1.63 (0.40)	0.022
MF-R	1.18 (0.60)	2.14 (0.66)	0.004
MF-L	0.72 (0.70)	1.74 (0.42)	0.001

cLBP: chronic low back pain; L: left; R: right; TVA: transversus abdominis; MF: multifidus; CoG: center of gravity.

right-handed cLBP subjects might preferentially recruit their right-sided muscles in performing complex functional tasks, resulting in the TVA and MF muscles being discrete in the representative areas of the left cerebral hemisphere. Additional research is warranted to investigate cortical representation recruitment of left-handed cLBP subjects.

In addition, our results showed that the main reason for the relatively discrete change between the representation of

Table 3: TVA and MF muscle dominant hemispheres in healthy and cLBP subjects.

Subjects	Healthy TVA	Healthy MF	cLBP TVA	cLBP MF
1	-0.47	0.09	0.08	-0.56
2	0.38	0.71	0.38	0.33
3	0.60	-0.60	0.23	0.86
4	-0.47	0.00	0.07	0.00
5	-0.50	0.20	0.45	0.00
6	0.08	-0.43	0.00	-0.20
7	-0.22	0.33	0.85	0.25
8	0.43	0.78	0.08	0.64
9	0.43	0.00	-0.24	0.00
10	0.25	0.09	-0.33	-0.65
11	-0.47	0.71	0.00	/
Right-sided (%)	36.4	18.2	18.2	30.0
Left-sided (%)	45.5	45.5	36.4	40.0
Mutual (%)	18.2	36.4	45.5	30.0

cLBP: chronic low back pain; TVA: transversus abdominis; MF: multifidus.

two muscles in the cLBP group was that the shift of the MF muscle was farther away than that of the TVA muscle. This may suggest that the MF muscle disorder in cLBP patients is more prominent than that of TVA. Two early studies [12, 33] reported the clinical importance of the prescription of MF muscle activation for patients with LBP, instead of TVA muscle activation as part of the core stability exercise program. Additionally, atrophic changes of MF were reported in about 77–80% of LBP cases, especially at the L5–S1 level [34], which is the same EMG site of MF in our study. The current study revealed only the changes in the representation of the two muscles in the motor cortex of cLBP patients. Therefore, further studies must link anatomical asymmetries to brain function and behavior using task-related fMRI or ERPs.

4.3. Interhemispheric Asymmetry of TVA and MF Muscles. Our study found that there was lateralization to the left (both TVA and MF: 45.5%) in healthy subjects, while there was a trend of mutual symmetry (TVA: 45.5%, MF: 30.0%) in the cLBP groups (Table 3). This hemispheric asymmetry occurs not only in the trunk muscles but also in the swallowing muscles [16], pectoralis major, and latissimus dorsi [34, 35]. Hamdy et al. [16] suggested that there was no consistent relationship between handedness and lateralization. Differences between the left and right hemispheres might be part of a general left-right asymmetry of the motor system in the healthy subjects [36] and might be dependent on the same repetitive event or factors that break body asymmetry. The asymmetry is potentially the neural basis of pathokinesiology of cLBP [35].

4.4. Limitations. Despite our important observations, our study had some limitations. Firstly, the subjects in the two groups tended to be younger (mean 28 years). Changes in brain parenchyma are associated with age [37]. Additional studies can expand the sample size and classify age-related differences to investigate the excitation of trunk muscle cortical representations. Moreover, the present study did not consider left-handed subjects, and the current results cannot be applied to left-handed individuals. Future studies are recommended to investigate the relationship between TVA and MF muscles in the motor cortex in left-handed individuals. Lastly, our conclusion is based only on evidence from TMS, but other brain imaging techniques (such as fMRI and EEG) have not yet found it. In future studies, we will further study the brain network relationship between TVA and MF muscles by combining brain function and behavior with task-related fMRI or EEG [38, 39]. The present study adopted the figure-of-eight coil to provide a focalized stimulation on the contralateral motor cortex to map the trunk muscles responses. However, other scholars proposed that the double-corn coil may be more appropriate since it induces contralateral and ipsilateral responses of the trunk muscles consistently [40]. Further research may be warranted to compare the muscle responses elicited by the two different types of coil.

5. Conclusions

The cortical representations of TVA and MF muscles were closely organized in the right and left motor cortices and leftward asymmetric in the healthy group. However, the cortical representations of TVA and MF muscles were discretely organized in the left motor cortex and mutually symmetrical in the cLBP group. Brain mapping is fundamental to the understanding of brain organization and function in cLBP patients. Our findings might reveal the relationship between cLBP and cortical reorganization and muscular system dysfunction, providing additional support for the neural basis of pathokinesiology and clinical treatment of cLBP.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

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