Research Article

Effect of vocal respiratory training on respiratory function and respiratory neural plasticity in patients with cervical spinal cord injury: a randomized controlled trial

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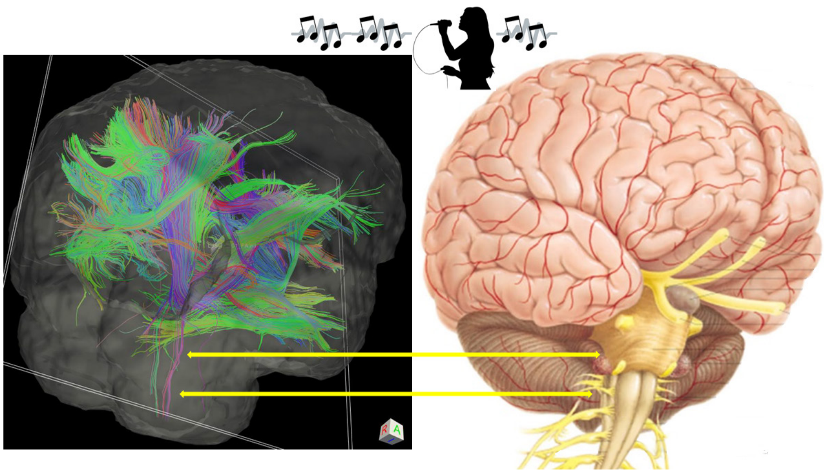
Graphical Abstract *Singing has a positive effect on respiratory central nerve fiber bundles in cervical spinal cord injury patients*



Abstract

In previous studies, researchers have used singing to treat respiratory function in patients with spinal cord injury. However, few studies have examined the way in which vocal training affects respiratory neural plasticity in patients with spinal cord injury. Vocal respiratory training (VRT) is a type of vocal muscle-related treatment that is often a component of music therapy (MT) and focuses on strengthening respiratory muscles and improving lung function. In this randomized controlled study, we analyzed the therapeutic effects of VRT on respiratory dysfunction at 3 months after cervical spinal cord injury. Of an initial group of 37 patients, 26 completed the music therapy intervention, which comprised five 30-minute sessions per week for 12 weeks. The intervention group (n = 13) received VRT training delivered by professional certified music therapists. The control group (n = 13) received respiratory physical therapy delivered by professional physical therapists. Compared with the control group, we observed a substantial increase in respiratory function in the intervention group after the 12-week intervention. Further, the nerve fiber bundles in the respiratory center in the medulla exhibited a trend towards increased diversification, with an increased number, path length, thickness, and density of nerve fiber bundles. These findings provide strong evidence for the effect of music therapeutic VRT on neural plasticity. This study was approved by the Ethics Committee of China Rehabilitation Research Center (approval No. 2020-013-1) on April 1, 2020, and was registered with the Chinese Clinical Trial Registry (registration No. ChiCTR2000037871) on September 2, 2020.

Key Words: cervical spinal cord injury; music therapy; neural plasticity; respiratory center; respiratory function; vocal respiratory training

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Introduction

Spinal cord injury (SCI) refers to structural and functional injury of the spinal cord caused by various pathogenic factors (e.g., trauma, inflammation, tumor), resulting in spinal nerve dysfunction below the level of injury (Brown et al., 2006; Krassioukov, 2009; Wei et al., 2019; Choi et al., 2021). SCI can lead to serious physical disability, and thus can affect patient quality of life and social participation, as well as carry a heavy burden for families and society (Brown et al., 2006). According to the statistics collected by the World Health Organization, 250,000 to 500,000 people globally acquire SCI every year, and the probability of paraplegia is 70% (The American Spinal Injury Association, 2000). When a spinal cord is injured, the nerve supply to the muscle becomes impaired, paralyzed, or damaged, and symptoms depend on the severity of the injury and the location on the spinal cord. Damage at a higher point in the body corresponds with a more severe loss of function. After SCI, complete or partial paralysis of the respiratory muscles can affect respiratory function. Respiratory dysfunction caused by cervical SCI (CSCI) is the main cause of disease and death in SCI patients (Cosortium for Spinal Cord Medicine, 2005; Baydur and Sassoon, 2010; Hoh et al., 2013; Hachmann et al., 2017). The proportion of death caused by respiratory complications after SCI has been reported to be as high as 91.66% (Gee et al., 2019), making respiratory complications a primary cause of death in patients with SCI.

According to the classification system used by the American Spinal Injury Association (ASIA) (Casha and Christie, 2011), patients with grade A, which refers to complete SCI; and grade B, which refers to incomplete SCI, are likely to develop post-injury respiratory dysfunction (Berlowitz et al., 2016). The degree of dysfunction depends on the level and extent of injury, and function can gradually deteriorate over time following SCI. Studies have shown that damage at a level higher than motor neurons (C3–5) can result in complete paralysis of the inspiratory and expiratory muscles, making these patients dependent on mechanical ventilation or nerve stimulation for respiratory function (Javed and Bogdanov, 2010). In general, patients with C1–2 injuries have severe respiratory muscle weakness and require a tracheotomy or mechanical ventilation with a ventilator. In contrast, patients with C3–4 injury generally have an impaired phrenic nerve, and therefore reduced functional tidal volume and vital capacity (VC), but can breathe autonomically without the use of a ventilator. In patients with C5 injury, diaphragm motor function remains intact, but paralysis of the intercostal muscles and abdominal muscles can lead to a decline in lung function, resulting in shallow breathing and weak sputum (Berlowitz et al., 2016). A total or partial loss of respiratory muscle function can lead to a decrease in inspiratory and expiratory muscle strength. Patients with respiratory dysfunction after CSCI usually suffer from respiratory muscle weakness or paralysis, which leads to a decrease in lung and chest wall compliance (Hoh et al., 2013; Berlowitz et al., 2016; Alajam et al., 2019). Decreased chest wall compliance can elicit changes the mechanical properties of the lungs, which can take place rapidly during low-capacity ventilation (Mueller et al., 2013). The most common complications associated with decreased respiratory function are forced VC reduction, decreased total lung capacity (TLC), increased residential vital (RV), increased airway resistance, difficulty breathing deeply, weak or ineffective cough, and increased sputum and/or symptoms of respiratory infection. These complications can lead to respiratory damage, resulting in chronic respiratory dysfunction (Liaw et al., 2000; MacBean et al., 2006).

Respiratory muscle training is often used clinically to improve respiratory function and respiratory endurance in patients with CSCI (Watson and Hixon, 2001). Studies have reported an obvious enhancement in respiratory function in patients with SCI after respiratory training (Tamplin et al., 2011, 2013; Chiou et al., 2020; Zhang et al., 2021). At present, patients with respiratory dysfunction after SCI usually undergo respiratory muscle training as part of physical therapy to help them exercise their respiratory muscles and improve lung function. This effective physical therapy method is focused on increasing muscle tone and strength, and has specific rehabilitation applications in terms of strengthening breathing muscles and improving lung capacity. Although such exercises are effective in terms of respiratory recovery, patients often report finding them tedious, uninteresting, and difficult to adhere to, as they are without immediate rewarding feedback (Chiou et al., 2020). Further, the outcome is directly linked to the amount of time spent exercising, and function often declines when training stops. To address this, clinicians and researchers have continuously investigated improved exercise techniques. Vocal respiratory training (VRT) is often delivered as a component of vocal coaching in music therapy (MT). In this therapeutic method, patients with respiratory dysfunction after SCI engage in singing exercises to enhance respiratory function (Tamplin et al., 2011, 2013; No authors listed, 2016; Zhang et al., 2021). During VRT, patients are taught to combine chest-type breathing and abdominal-type breathing in the process of respiration, and to use specific methods of inhalation and exhalation involving the chest, abdominal cavity, and diaphragm to increase atmospheric capacity, accelerate the exchange of air flow, and enhance respiratory function. After patients are helped to adopt good respiratory habits, they are encouraged to produce sound at a natural volume using the resonance or co-vibration of the diaphragm, chest, organs, and pharynx cavity and to increase the timbre of the resonant cavity by coordinating respiration so as to avoid excessive breathing and improve the sound quality. Vocal coaching and singing are closely related to respiratory training. Singing requires careful breathing control because it involves intense contraction of the diaphragm during deep inhalation, followed by closure of the vocal cords during exhalation to control the contraction of the diaphragm and other exhalation muscles. Therefore, singing exercises involving core respiratory muscle groups can play a positive role in respiratory recovery in patients with SCI (Tamplin et al., 2011; No authors listed, 2016; Zhang et al., 2021).

Studies have examined the use of singing to help patients with CSCI to train their respiratory muscles, with positive results (Tamplin et al., 2011, 2013). However, previous studies have only focused on singing activities, and not specifically on the application of VRT. Some scholars have compared the effects of specific vocal coaching techniques, oral motor and respiratory exercise, and vocal intonation therapy (Zhang et al., 2021) on respiratory function in patients with CSCI, and others reported that specific vocal techniques had significant effects on the results of a respiratory function test (Tamplin et al., 2013). However, these trials only focused on changes in functional measurements, and not on the neuroplasticity caused by the vocal musical behavioral changes. In this study, we conducted a VRT intervention in patients with respiratory dysfunction after SCI, and compared the effects with those of respiratory physical therapy. We had two main research goals. The first was to compare VRT with respiratory physiotherapy in terms of the speed and extent of functional recovery, and determine which respiratory measures produced the most positive outcomes. The second was to use diffusion tensor imaging (DTI) to determine which patient group exhibited more positive changes in the nerve fiber tracts in the respiratory center, so as to further assess the value of MT for treating respiratory dysfunction after SCI.

Subjects and Methods

This randomized controlled study was approved by the Ethics Committee of Capital Medical University (approval No. 2020-013-1) on April 1, 2020, and all of the patients and their legal guardians signed consent forms before engaging in the study. The clinical trial was registered with the Chinese Clinical Trial Registry (No. ChiCTR2000037871) on September 2, 2020. The study protocol and execution were in compliance with the principles included in the *Declaration of Helsinki*. The writing and editing of the article were performed in accordance with the CONsolidated Standards Of Reporting Trials (CONSORT) Statement (**Additional File 1**).

Subjects
Thirty-seven patients were recruited from Capital Medical University. The inclusion criteria for the study were: 1) diagnosed with SCI, ASIA grade A or B (Thaut and Hoemberg, 2014), SCI duration of at least three months (inclusive), currently hospitalized; 2) age 18–60 years; 3) native Chinese speaker; 4) absence of postural hypotension; 5) tolerance for sitting 0.5 hours; 6) stable medical treatments; 7) no prior professional musical experience. The exclusion criteria were as follows: 1) severe neurological disease, severe respiratory disease; 2) cognitive dysfunction, Mini-Mental State Examination (Folstein et al., 1975) score ≤ 17 for illiteracy or ≤ 20 for primary school; 3) epilepsy, arrhythmia, and other serious diseases that could limit the amount of time a participant could remain seated for training. Withdrawal and termination criteria: timely termination upon discharge from the hospital or withdrawal midway. Eleven subjects withdrew because they were not meeting the inclusion criteria, two refused to participate, and two left the study for undisclosed reasons. The sample size was calculated according to the formula ($n = Z^2 \cdot \sigma^2 / d^2$). The minimum number of subjects needed to satisfy the statistical power of the analysis was 18. Therefore, the minimal sample number met the requirements (n = 26). **Table 1** shows the participant demographic characteristics.

Table 1 | Participant demographic characteristics

	Intervention group	Control group	*P*-value
Total number	13	13	> 0.05
Sex (*n*)			
Male	10	11	> 0.05
Female	3	2	> 0.05
Age (yr)	39.31 ± 17.87	40.54 ± 19.88	> 0.05
Months since injury	6.03 ± 4.57	5.48 ± 3.71	> 0.05
Height (cm)	174.00 ± 10.44	166.62 ± 9.97	> 0.05
Weight (kg)	67.46 ± 12.96	62.46 ± 16.04	> 0.05
Body mass index (kg/m^2)	22.23 ± 3.63	22.35 ± 4.77	> 0.05
American Spinal Injury Association classification			
A	6	5	> 0.05
B	7	8	> 0.05
Injury level grading			
C4	8	7	> 0.05
C5	5	6	> 0.05

Data are expressed as number in total number, gender, and AISA classification, and were analyzed by paired *t*-test. The other data are described as the mean ± SD, and were analyzed by paired *t*-test. Intervention group: Vocal respiratory training group; control group: respiratory physiotherapy group.

Study design
The subjects were divided into a VRT intervention group (n = 13) and a control group (n = 13), who received respiratory physiotherapy. Data were collected using lung function tests at baseline (T0), intermediately after the first session (T1), and at the end of the experimental period (T2). At baseline (T0) and at the end of the experiment (T2), we used functional magnetic resonance imaging-DTI to assess changes in respiratory central nerve fiber bundles.

Trial procedure
After being approved for study inclusion, the subjects were screened by SCI specialists. Patients with CSCI with ASIA grade A or B, C4 and C5 injuries, and with respiratory dysfunction were referred to the MT department. Researchers evaluated the subjects and designed the intervention based on the inclusion and exclusion criteria. After the subjects were confirmed, the researchers sent them a research invitation to explain the purpose, procedures, and interests of the study, as well as inform them of the risks, confidentiality policy, and participant rights. After the subjects signed the informed consent form, the researchers randomly assigned the subjects into one of the two groups according to a computer-generated sequence (Excel 2013, Microsoft office software, Seattle, WD, USA). The participants in the intervention group received one-on-one VRT training, conducted by a professional music therapist, for 12 weeks (0.5 hours/session, five sessions per week). The control group received bedside training delivered by a respiratory physiotherapist for 12 weeks (0.5 hours/session, five sessions per week). **Figure 1** shows the study procedure.

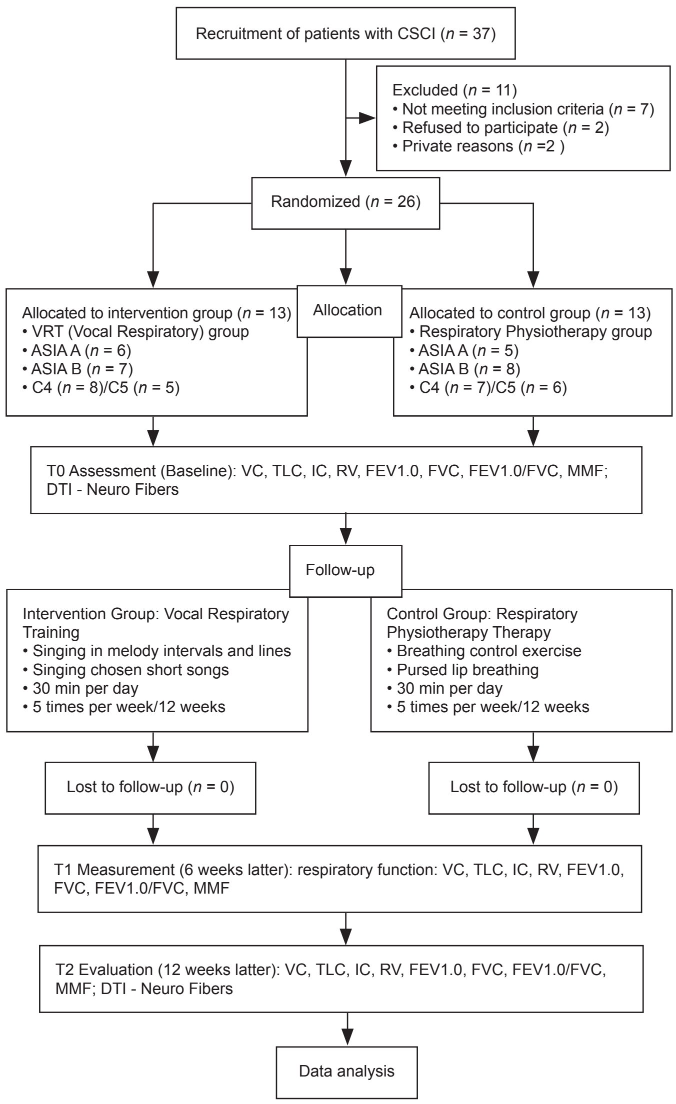

Figure 1 | Flow chart of participant recruitment and group allocation.
ASIA: American Spinal Cord Injury Association; CSCI: cervical spinal cord injury; DTI: diffusion tensor imaging; FEV1.0: forced expiratory volume in one second; FVC: forced vital capacity; IC: inspiratory capacity; MMF: maximal mid-expiratory flow rate; RV: residual capacity; T0: baseline; T1: intermediate (6 weeks) point of the experiment; T2: the end (12 weeks) of the experiment; TLC: total lung capacity; VC: vital capacity; VRT: voice breathing training.

Interventions

Once the group allocation was complete, a detailed intervention plan was developed. In the intervention group, a music therapist provided each subject with one-on-one VRT training based on MT. Each half-hour VRT training session had the following components. For the first 10 minutes, the music therapist led the patient in singing a short melody (**Additional Figure 1**, subsection 1–24), and then guided the patient to sing the short melody using a staccato singing method to enhance abdominal muscle strength. In the next 10 minutes, the music therapist guided the patient in singing vowels with different tonal melody lines (**Additional Figure 2**, bars 1–40). In the last 10 minutes, the patient was instructed to sing specific songs. The songs were selected according to grades 1–3 of the Chinese Musicians Association Vocal Test (Xu and Gong, 2014). The control group received one-on-one training from respiratory physiotherapists, including respiratory control exercises, shrinking lips abdominal breathing, and respiratory physiotherapy (No authors listed, 2016). The treatment sessions in both groups were 0.5 hours long, with five sessions per week for 12 weeks. Except for VRT training and respiratory physiotherapy training, the two groups of patients had the same medication regimens, routine care, and medical support.

Measurements

The two groups of subjects were assessed before each intervention, including at baseline (T0), a mid-term evaluation (T1) after 6 weeks, and a final evaluation after 12 weeks (T2). The assessments included a test of respiratory function and DTI, which was included in the magnetic resonance imaging examination at T0 and T2. During the evaluation and training process, no subjects used abdominal adhesives or other tools that could affect the accuracy of the lung function test, and all of the participants were able to tolerate the magnetic resonance imaging-DTI test.

Respiratory function tests

The tests of respiratory function were conducted according to the guidelines of the American Thoracic Society (Clini et al., 2018; Lemos et al., 2020), and were modified to incorporate the limitations associated with SCI (Black and Hyatt, 1971; Ashba et al., 1993; Kelley et al., 2003). Respiratory function tests were accomplished using a lung function assessment instrument (FGC-A$^+$ Anke Biotechnology Group Co., Ltd., Hefei, Anhui Province, China). The vital capacity (VC), total lung capacity (TLC), inspiratory capacity (IC), residual volume (RV), forced expiratory volume in one second (FEV1.0), and forced vital capacity (FVC) were evaluated in L; the maximal mid-expiratory flow rate (MMF) was evaluated by L/s, and FEV1.0/FVC was evaluated as a percentage (Xiangshangdegongchengshi, 2019). At the same time, the maximum inspiratory volume, maximum expiratory volume, ventilation function, and upper respiratory airway function were evaluated using a spirometer.

DTI

DTI, a special form of magnetic resonance imaging, is a new method for evaluating brain structure (Baker, 2000). DTI creates a map of the connections between nerve cells and illuminates the network structure by tracking the direction of water molecules in the brain (Xu and Gong, 2014). We acquired DTI data using a single-shot, spin-echo, echo-planar pulse sequence (Calamuneri et al., 2018). Magnetic resonance imaging was performed with the patient in a supine position. We used a 3.0T magnetic resonance imaging scanner (Philips Ingenia, Best, The Netherlands) with an 8-channel phase-array head coil. Tight but comfortable foam padding was used to minimize head movement, and earplugs were used to reduce exposure to the noise caused by scanning. Sagittal three-dimensional structural T1-weighted images were acquired with the following parameters: repetition time (TR)/echo time (TE) = 7.0/3.2 ms; flip angle = 7°; number of slices = 192; slice thickness = 1 mm; field-of-view (FOV) = 256 × 256 mm^2; and matrix = 256 × 256; 1 mm^3 isotropic resolution. The resting-state functional data were obtained using a single echo-planer imaging sequence with the following parameters: TR = 2000 ms, TE = 30 ms, slice thickness = 3.5 mm, number of slices = 32, FOV = 224 × 224 mm^2, matrix size = 64 × 64, interslice gap = 0.8 mm, flip angle = 90°. TR = 10,624 ms, TE = 89 ms, flip angle = 90°, one image with b = 0 s/mm^2 and 32 independent directions with b = 1000 s/mm^2, thickness slices = 2 mm, slice gap = 0, FOV = 224 × 224 mm^2, matrix size = 112 × 112 (Xu and Gong, 2014).

We used PANDA (Pipeline for Analyzing brain Diffusion images) software, based on MATLAB (http://www.nitrc.org/projects/panda), to correct the DTI original images for head motion. After the patient's T1-weighted image was mapped to the corresponding = 0 s/b DTI images, we created calibrated images in DTI space (Fan et al., 2016). According to the anatomical calibration template of the Institute of Automation, Chinese Academy of Sciences, the brain was divided into 246 regions (Crossley et al., 2017) to obtain the network nodes of the brain structure. The number of fibers in a pair of brain intervals was the threshold T (Lumsden, 1923), and a weighting matrix of 246 × 246 was obtained. Fiber number (FN) > T denotes the presence of fibrous connections in the brain region, defined as 1, and this was otherwise defined as 0. We converted the entitlement matrix into a binary matrix, and generated a model of the structural network of the entire brain. The characteristic parameters of the brain structural network at the FN threshold point were calculated respectively. There are three colors in the 3D DTI diagram: red indicates the left and right direction, green indicates the front and back direction, and blue represents the up and down direction. Darker colors indicate a higher degree of anisotropy.

Statistical analysis

The data from the two groups were collected at T0, T1, and T2. If the data had a normal distribution, the time and interaction effects between the groups were analyzed using a repeated measures two-way analysis of variance followed by Sidak's multiple comparisons test using SPSS 22.0 (IBM, Armonk, NY, USA) at T0, T1, and T2.

Results

Effectiveness of VRT on respiratory function in CSCI patients

We measured the respiratory function of the two groups at T0–2. VC (F = 7.100, P = 0.0095), TLC (F = 4.122, P = 0.0460), IC (F = 9.503, P = 0.0029), RV (F = 7.714, P = 0.0499), FEV1.0 (F = 5.708, P = 0.0195), FVC (F = 4.720, P = 0.0331), FEV1.0/FVC (F = 40.15, P = 0.0001), and MMF (F = 9.964, P = 0.0022) were higher in the intervention group at T2 compared with those in the control group. In the intervention group, we observed a significant difference between T0 and T2 in terms of IC (F = 3.842, P = 0.0260), FEV1.0/FVC (F = 10.29, P = 0.0001), and MMF (F = 7.393, P = 0.0007). The results of the two groups are shown in **Figure 2**.

Effectiveness of VRT on neural plasticity in the respiratory center in CSCI patients

According to the 246 brain regions obtained after the PANDA analysis, the fractional anisotraphy (FA), FN, and path length of the medial thalamic nucleus, nucleus anterior thalami, nuclei lateralis thalami, dorsal ventral thalamus nucleus, ventral posterior nucleus of thalamus, spinothalamic tract, posterior parietal lobe of thalamus, lateral frontal lobe of the thalamus, ventral cortex of the occipital lobe, gyrus lingualis, rostral area of the cuneate gyrus, ventromedial parietooccipital sulcus, inferior occipital gyrus, inferior gyri occipitales superiores, lateral gyri occipitales superiores, and lateral occipital cortex were markedly increased in the VRT group compared with the control group (**Figure 3**).

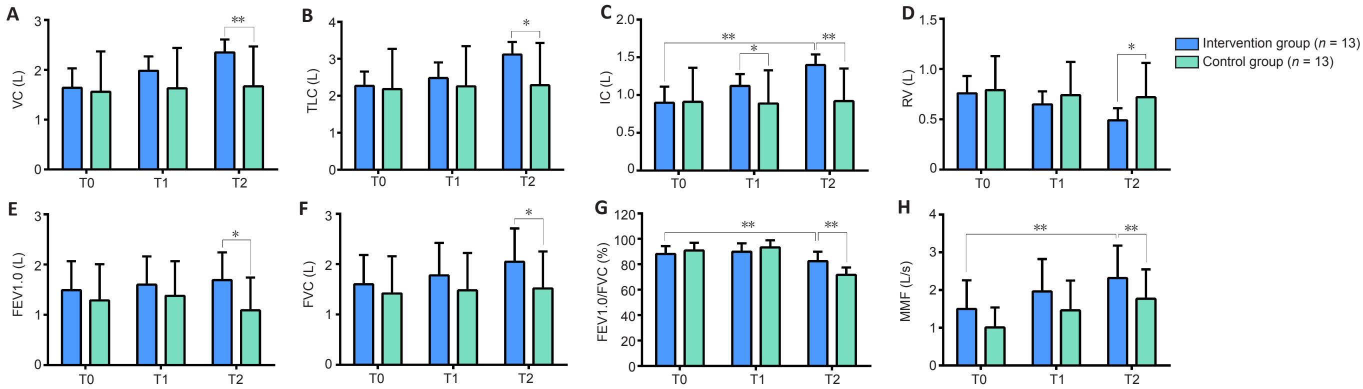

Figure 2 | **Respiratory function in cervical spinal cord injury patients who underwent voice breathing training *versus* respiratory physical therapy.**
Intervention group: Vocal respiratory training group; Control group: respiratory physical therapy group. (A–H) VC, TLC, IC, RV, FEV1.0, FVC, FEV1.0/FVC, and MMF. Original data are shown in Additional File 2. Data are described as the mean ± SD (n = 13). *P < 0.05, **P < 0.01 (repeated measures two-way analysis of variance followed by Sidak's multiple comparisons test). FEV1.0: Forced expiratory volume in one second; FVC: forced vital capacity; IC: inspiratory capacity; MMF: maximal mid-expiratory flow rate; RV: residual capacity; T0: baseline; T1: intermediate (6 weeks) point of the experiment; T2: the end (12 weeks) of the experiment; TLC: total lung capacity; VC: vital capacity.

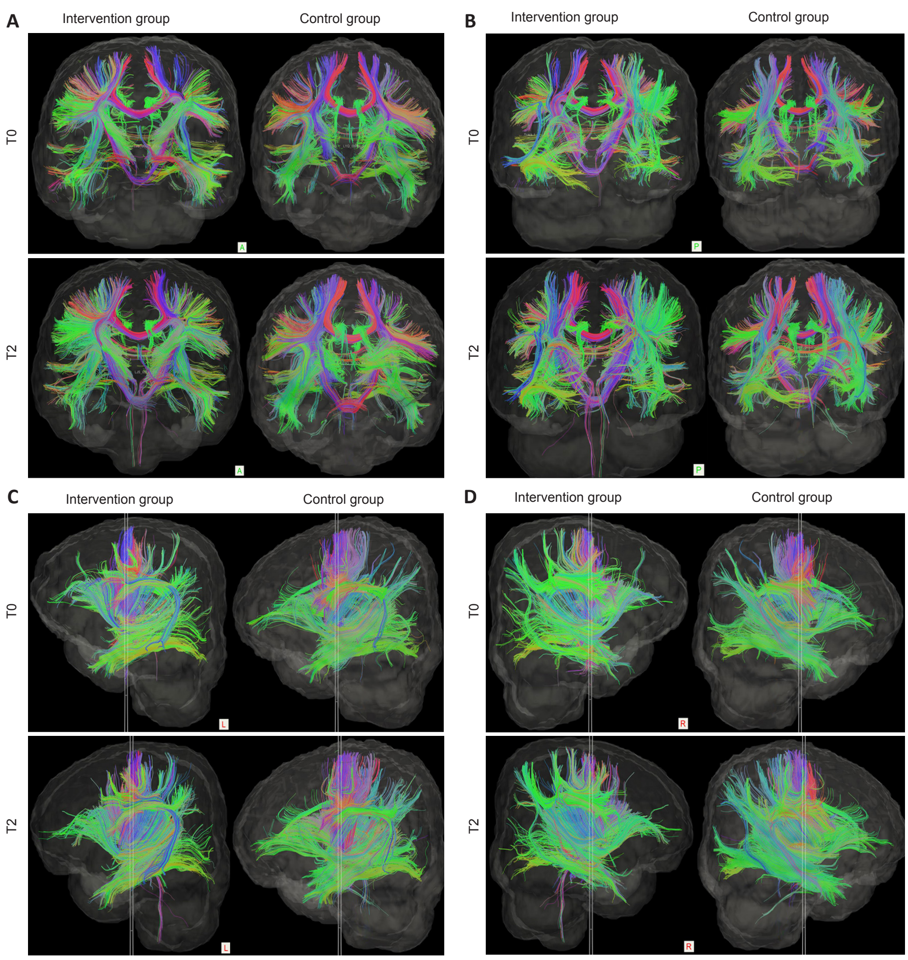

Figure 3 | **Neural plasticity in the respiratory center on the anterior-posterior and left-right planes in patients with cervical spinal cord injury treated with vocal respiratory training *versus* respiratory physical therapy.**
Intervention group: vocal respiratory therapy group; control group: respiratory physiotherapy group. (A) Anterior plane. (B) Posterior plane. (C) Left plane. (D) Right plane. There was an obvious difference between the intervention group and the control group. The diversity of fiber anisotropy, the density of fibers, and the length of the neural fibers in the intervention group approached those in the control group after 12 weeks of vocal respiratory training. A: Anterior plane; P: posterior plane; L: left plane. R: right plane; T0: baseline; T2: the end (12 weeks) of the experiment.

Discussion

Conventionally, treatment of respiratory dysfunction in patients with severe CSCI has focused on tracheotomy, mechanical ventilation with the help of a ventilator, or surgical installation of a phrenic nerve pacemaker for recovery. CSCI usually involves the region of the respiratory center that is located above the spinal cord, which is located in the medulla. Extensive paralysis of the respiratory muscles not only causes sputum expectoration and pneumonia, but can also lead to respiratory complications, and in severe cases, death. Therefore, effective respiratory function rehabilitation exercises are needed in the early stage of CSCI (Bach, 2006; Tamplin et al., 2011, 2013; Hoh et al., 2013; Berlowitz et al., 2016; Gee et al., 2019; Chiou et al., 2020; Zhang et al., 2021). In this study, we found that VRT had a positive effect on the respiratory central nerve fiber bundles in the MT group. Our results showed that the patients in the intervention group not only exhibited substantial improvements in respiratory function compared with the control group, but that they also had increased plasticity in the respiratory central nerve fiber bundles.

Respiratory function

VRT training is more detailed and step-by-step compared with respiratory physiotherapy. The vowel practice in VRT not only increased breath flow after a single inhalation, but also increased the VC, FEV1.0, and MMF. The melodic practice in which notes of different lengths are sung (**Additional Figures 1** and **2** show a spectrum example) increases the degree to which the diaphragm participates in inspiratory movements, expands the lung capacity, and is very effective for respiratory rehabilitation after SCI.

Effect of VRT on neuroplasticity in the respiratory central nerve fiber bundles FA, FN, and path length in patients with CSCI

FA

FA refers to the degree of anisotropy of nerve fiber bundles, which reflects the degree of molecular displacement in space, and is related to the direction of the tissue (Baker, 2000). We found that the FA-weighted structure network for the intervention group showed a clear trend of diversification. We specifically observed subcortical nuclei of motor neurons in the dorsal respiratory group and the ventral respiratory group, the ventromedial nucleus, the premotor area of the thalamus, the posterior parietal lobe of the thalamus, the occipital lobe of the thalamus, the temporal tail of the thalamus, and the spinothalamic tract. This increase in the nodes in the above areas indicates that they had become more active in the neural network involved in respiratory movement. The thalamus is located in the dorsal and ventral parts of the diencephalon, and has extensive connections with the spinal cord, brainstem, and cerebellum.

The medulla is part of the respiratory regulation center (Li et al., 2010). Whether it is practicing reading poetry or performing conscious breathing training before singing, a high-level nerve center in the cerebral cortex participates in these activities. A temporal lobe region that is implicated in the primary and secondary auditory cortex (Broadmannn areas 41, 42) processes the input of music and auditory signals, and an area responsible for the output of oral movements performs voice expressive output (Broadmannn areas 44, 45). Patients in the intervention group performed conscious respiratory motor nerve signal commands, issued by the frontal cortex during singing activities, to accomplish integrated exercises of multiple brain regions and multiple nerve fiber bundles. This promoted the activity of the respiratory central nerve fiber bundles. The molecular orientation increased the participation of cortical activities to a certain extent. Therefore, the observed increase in the degree to which the fiber bundles had multiple nodes in the FA from the intervention group indicated that the fiber bundles associated with high-level neuroregulation related to respiration showed a trend towards diversification.

FN and path length

We used FN and path length to analyze the similarities and differences in the number of nerve fibers and the path length between the intervention and control groups, as per previous methods (Baker, 2000; Zhang et al., 2015). From the analysis of the FN of the intervention group, the areas of increased respiratory activity related to singing in the FN-weighted structural network were also mainly distributed in the subcortical nucleus, dorsal caudate area, medial prefrontal lobe of the thalamus, and posterior roof of the thalamus. Linguistic and larynx areas in the parietal lobe that are related to the movement of speech showed a corresponding enhancement in motor activity, which was likely because singing involves multi-level oral execution such as language vocabulary movement. These regions showed positive changes in the intervention group.

The activated brain areas associated with music were located in the middle and dorsolateral areas of the superior frontal gyrus, dorsal tail of the middle frontal gyrus, caudal area of the inferior frontal gyrus, orbital lobe gyrus, and dorsolateral caudal area of the central anterior gyrus. We also observed activation in the central and rostral areas of the temporal lobe, central posterior gyrus of the parietal lobule, dorsal medial parieto-occipital groove of the precuneus, and trunk area of the central posterior gyrus. Similarly, path length had the most concentrated manifestations in the subcortical nucleus, including the ventral caudate, globus pallidus, nucleus accumbens, ventromedial nucleus, and dorsolateral nucleus of the basal ganglia, as well as in the medial prefrontal lobe, temporal rostral and posterior parietal region of the thalamus, and lateral prefrontal lobes.

Compared with the control group, the intervention group has a significantly higher level of information exchange at nodes above the weighted structure network of the FN and path length, which had the same nerve fiber bundle distribution characteristics as in the FA. For both the number of fiber bundles in this brain area and the amount of growth, we observed accelerated global efficiency of neural signal processing in the brain network.

Limitations

This study was a small randomized controlled trial, and 11 subjects were excluded from the analysis. Thus, our results describe the outcome of one neurological music therapy technique for respiratory training in a small patient group. If a standard care group is added in future research, the results may be more accurate. A larger-scale study would be helpful in accurately determining the treatment effect.

Implications for clinical practice

Based on the above respiratory function test and DTI imaging results, patients with CSCI who participated in VRT training activities exhibited clear functional activation of the sensorimotor area of the cerebral cortex when they consciously regulated respiratory muscle movement during singing and MT activities. CSI patients who attended VRT training learned to send signal commands through the advanced cortex, consciously regulate skeletal muscle-respiratory muscle movement, mobilize involuntary muscles, and activate the autonomic nervous system, thus playing a positive role in promoting the regeneration and remodeling of nerve fiber bundles. Singing requires constant input of musical auditory stimuli and a regular segmentation process guided by rhythm. The patients who participated in the singing activities continuously adjusted their respiratory muscles and labioglossus muscles while the temporal lobe auditory cortex participated in auditory perceptual processing. Therefore, when the SCI patients in the intervention group participated in the singing activities, the nerve fibers in the frontotemporal lobe and subcortical nucleus were activated by higher cortical nerve signals. Thus, the interaction between cortical activity and respiratory center activity increased the density, width, and path length of nerve fiber bundles.

Conclusions

Our data indicate that VRT - MT can improve respiratory function and vocal function in patients with CSCI and that the neural regulation mechanisms of the respiratory center can be used as an effective basis for behavioral therapy. As a non-invasive and effective treatment, VRT is an important new treatment option for individuals with respiratory dysfunction after SCI.

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Additional files:
Additional Figure 1: *Music pieces bars 1–24.*
Additional Figure 2: *Music pieces bars 1–40.*
Additional file 1: *CONSORT checklist.*
Additional file 2: *Original data for Figure 2.*
Additional file 3: *Open peer review reports 1 and 2.*

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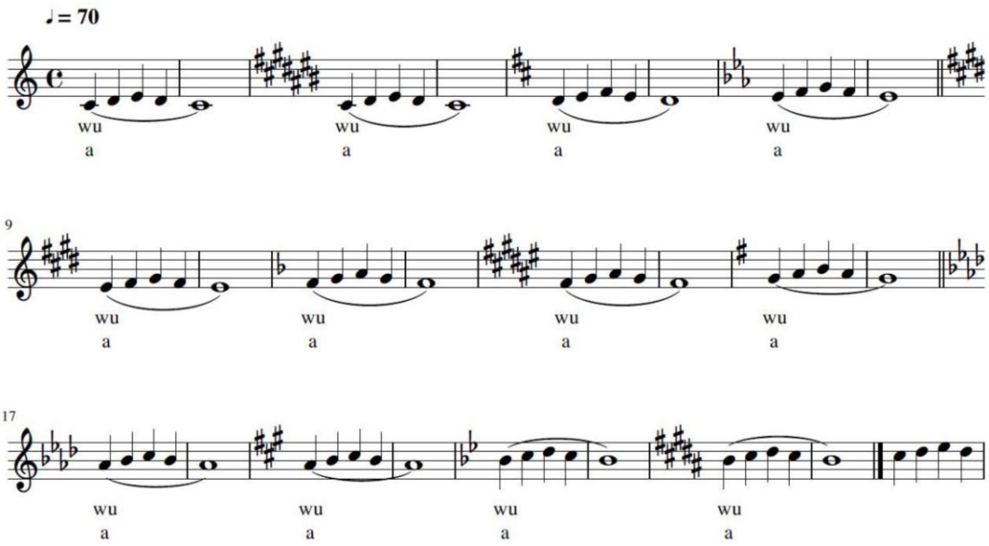

Additional Figure 1 Music pieces bars 1-24.

Additional Figure 2 Music pieces bars 1-40