Working-memory training improves developmental dyslexia in Chinese children*

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Abstract
Although plasticity in the neural system underlies working memory, and working memory can be improved by training, there is thus far no evidence that children with developmental dyslexia can benefit from working-memory training. In the present study, thirty dyslexic children aged 8–11 years were recruited from an elementary school in Wuhan, China. They received working-memory training, including training in visuospatial memory, verbal memory, and central executive tasks. The difficulty of the tasks was adjusted based on the performance of each subject, and the training sessions lasted 40 minutes per day, for 5 weeks. The results showed that working-memory training significantly enhanced performance on the nontrained working memory tasks such as the visuospatial, the verbal domains, and central executive tasks in children with developmental dyslexia. More importantly, the visual rhyming task and reading fluency task were also significantly improved by training. Progress on working memory measures was related to changes in reading skills. These experimental findings indicate that working memory is a pivotal factor in reading development among children with developmental dyslexia, and interventions to improve working memory may help dyslexic children to become more proficient in reading.

Key Words
neural regeneration; neurorehabilitation; developmental dyslexia; working memory; training; visuospatial memory; verbal memory; central executive task; visual rhyming task; reading fluency task; Chinese children; brain function; grants-supported paper; photographs-containing paper; neuroregeneration

Research Highlights
(1) Dyslexic children were trained on working memory tasks, including the visuospatial, verbal, and central executive domains.
(2) Working memory in children with developmental dyslexia can be improved through training.

INTRODUCTION
Developmental dyslexia is the most common learning disability, and is characterized by low reading abilities in children who have adequate intelligence, typical schooling, and sufficient sociocultural opportunities¹-². Many studies have shown that dyslexic children benefit from early intervention programs focusing on orthographic or morphological spelling treatments³⁴. However, some children do not respond to such programs. These improvements are more difficult to achieve for fluency than for accuracy⁵. Numerous studies have examined whether dyslexia involves deficits in sub-systems of working memory, such as phonological loops, visuospatial sketchpads, and central
executive functioning\(^6\)\(^-\)\(^9\). Furthermore, a multidisciplinary approach showed that a unifying theoretical framework for three working memory components may provide a system perspective for discussing past and present findings in a 12-year research program that point to heterogeneity in the neural basis and behavioral expression of dyslexia\(^1\(^0\). Recent studies found that children with attention-deficit/ hyperactivity disorder or with learning disabilities may experience beneficial effects from working-memory training\(^1\(^1\)\(^-\)\(^1\(^4\). Moreover, other recent data indicated working memory and literacy measures in adult dyslexic readers can be improved by working-memory training\(^1\(^9\). However, to date, there is no evidence that training on any other regimen yields increased working memory in children with developmental dyslexia. We hypothesized that working memory abilities in children with developmental dyslexia would improve following training, and these improvements would have a positive effect on the children’s reading skills.

**RESULTS**

Quantitative analysis of experimental subjects and baseline data

Thirty developmentally dyslexic children were randomly divided into treatment group (11 males and 4 females, 10.2 ± 0.8 years old) and control group (10 males and 5 females, 10.9 ± 0.9 years old), with no significant difference in the age and gender between two groups \((P > 0.05)\). The vocabulary test scores and intelligence quotient of children in treatment group were 2175 ± 309 words and 106 ± 7, while those in control group were 2208 ± 319 words and 108 ± 5, respectively, with no significant differences \((P > 0.05)\). Treatment group was engaged in training for 40 minutes a day and task difficulty was manipulated according to the completion of training. The control group was trained for 10 minutes a day, but the difficulty level was not interactively adjusted. All of them participated 5 weeks of training programs. Participant information is shown in Table 1.

| No. | Gender | Age (year) | Education (year) | Ethnicity | Vocabulary test scores (word) | Score of DCCC | Score of IQ |
|-----|--------|------------|------------------|----------|-------------------------------|---------------|------------|
| Treatment group | | | | | | | |
| 1   | Male   | 8          | 3                | Han      | 1 994                         | 74            | 99         |
| 2   | Female | 9          | 3                | Han      | 1 847                         | 75            | 110        |
| 3   | Male   | 8          | 3                | Han      | 1 937                         | 78            | 194        |
| 4   | Male   | 9          | 3                | Han      | 1 944                         | 79            | 101        |
| 5   | Male   | 9          | 4                | Han      | 2 018                         | 80            | 108        |
| 6   | Male   | 10         | 4                | Han      | 2 051                         | 73            | 103        |
| 7   | Female | 10         | 4                | Han      | 2 204                         | 75            | 108        |
| 8   | Female | 9          | 4                | Han      | 2 144                         | 78            | 102        |
| 9   | Female | 10         | 4                | Han      | 2 237                         | 79            | 97         |
| 10  | Male   | 11         | 5                | Han      | 2 318                         | 80            | 92         |
| 11  | Male   | 11         | 5                | Han      | 2 585                         | 83            | 100        |
| 12  | Male   | 10         | 5                | Han      | 2 620                         | 80            | 103        |
| 13  | Male   | 11         | 5                | Han      | 2 486                         | 85            | 115        |
| 14  | Male   | 11         | 5                | Han      | 2 585                         | 74            | 94         |
| 15  | Male   | 11         | 5                | Han      | 2 274                         | 77            | 108        |
| Control group | | | | | | | |
| 1   | Female | 9          | 3                | Han      | 1 868                         | 74            | 115        |
| 2   | Female | 8          | 3                | Han      | 1 872                         | 77            | 96         |
| 3   | Male   | 9          | 3                | Han      | 1 879                         | 77            | 118        |
| 4   | Female | 8          | 3                | Han      | 1 957                         | 74            | 111        |
| 5   | Male   | 9          | 3                | Han      | 2 147                         | 73            | 93         |
| 6   | Male   | 9          | 3                | Han      | 2 251                         | 74            | 106        |
| 7   | Female | 10         | 4                | Han      | 2 168                         | 77            | 98         |
| 8   | Male   | 10         | 4                | Han      | 2 187                         | 77            | 111        |
| 9   | Male   | 9          | 4                | Han      | 2 079                         | 82            | 122        |
| 10  | Male   | 10         | 4                | Han      | 2 545                         | 77            | 111        |
| 11  | Female | 11         | 5                | Han      | 2 362                         | 82            | 103        |
| 12  | Male   | 10         | 5                | Han      | 2 420                         | 83            | 115        |
| 13  | Male   | 11         | 5                | Han      | 2 423                         | 80            | 122        |
| 14  | Male   | 11         | 5                | Han      | 2 160                         | 85            | 88         |
| 15  | Male   | 10         | 5                | Han      | 2 286                         | 76            | 107        |

DCCC: Dyslexia Checklist for Chinese Children; IQ: intelligence quotient.
Working-memory training improved working memory in children with developmental dyslexia

There were no significant differences in baseline scores (T1) between the treatment group and the control group (P > 0.05). The effects of training in each group were tested by comparing the outcome scores after the training (T2) to the scores at T1. Performance on the four verbal working memory measures, the visuospatial working memory measure, and the central executive measure (time for completion of the Stroop task) was better at T2 than T1 in the treatment group (P < 0.05). Performance on two literacy measures (accuracy and reaction time in a visual rhyming task, P < 0.05; one-minute test of reading words, P < 0.01) was also better at T2 than T1 in the treatment group.

However, no significant differences were found for any measure in the control group (P > 0.10; Table 2).

Table 2  Task performance and effect sizes for the cognitive and literacy measures in the two groups

| Item                               | Control group | Treatment group | Group difference (t/P) | Effect sizes (d) |
|------------------------------------|---------------|-----------------|------------------------|-----------------|
|                                    | T1            | T2              | T1                    | T2              | Training-related (t/P) | Training-related (t/P) |                        |                        |
|                                    |               |                 |                        |                 |                       |                       |                        |                        |
| Verbal working memory              |               |                 |                        |                 |                       |                       |                        |                        |
| Digit span forward (scores)        | 9.47±1.88     | 9.60±1.80       | -0.12/0.845            | 9.38±2.32       | 10.23±1.92             | -2.67/0.020            | 0.459                   | 0.40                    |
| Digit span backward (scores)       | 4.00±1.51     | 4.20±1.32       | -0.39/0.702            | 4.08±1.61       | 5.00±1.58              | -3.21/0.008            | 0.040                   | 0.58                    |
| Word span task forward (scores)    | 9.86±2.03     | 10.07±1.79      | -0.29/0.777            | 10.00±2.00      | 11.23±1.54             | -2.48/0.029            | 0.065                   | 0.69                    |
| Word span task backward (scores)   | 3.33±1.35     | 3.47±1.18       | -0.23/0.776            | 3.15±1.41       | 4.46±1.61              | -2.85/0.015            | 0.007                   | 0.87                    |
| Visuospatial working memory        |               |                 |                        |                 |                       |                       |                        |                        |
| Corsi span task (scores)           | 3.40±0.83     | 3.60±0.74       | -0.69/0.490            | 3.40±1.24       | 4.87±0.83              | -3.79/0.001            | 0.002                   | 1.39                    |
| Central executive function         |               |                 |                        |                 |                       |                       |                        |                        |
| Stroop task                        |               |                 |                        |                 |                       |                       |                        |                        |
| Accuracy (scores)                  | 58.46±1.30    | 58.87±0.99      | -0.94/0.352            | 58.33±1.63      | 58.87±0.99             | -1.08/0.289            | 0.692                   | 0.40                    |
| Time for completion (second)       | 106.86±17.36  | 104.75±15.66    | 0.35/0.729             | 105.31±19.73    | 91.72±16.30            | 2.06/0.049             | 0.018                   | 0.75                    |
| Reading achievement                |               |                 |                        |                 |                       |                       |                        |                        |
| Visual rhyming task                |               |                 |                        |                 |                       |                       |                        |                        |
| Accuracy (scores)                  | 35.77±1.11    | 38.38±1.59      | -1.14/0.263            | 35.12±1.03      | 43.03±1.83             | -2.11/0.044            | 0.040                   | 0.77                    |
| Reaction time (ms)                 | 1108.16±139.78| 1045.02±104.71 | 1.39/0.173            | 1127.63±181.10 | 934.69±158.34          | 3.11/0.004             | 0.010                   | 1.13                    |
| One-minute tests of reading words  | 65.63±10.30   | 67.86±8.28      | -0.66/0.518            | 66.22±12.69     | 77.91±7.25             | -3.09/0.005            | 0.001                   | 1.13                    |

The range of scores in the digit span forward and digit span backward is 0–22 points, with higher scores indicating better verbal working memory. The range of scores in the word span task forward and word span task backward tasks is 0–18 points, with higher scores indicating better verbal working memory. The range of scores in the Corsi span task is 0–8 points, with higher scores indicating better visuospatial working memory. The range of accuracy scores in the Stroop task is 0–60 points, with higher scores indicating better central executive function. The range of accuracy scores in the visual rhyming task is 0–70 points, with higher scores indicating better reading skills.

Data are expressed as mean ± SD of fifteen participants in each group. Training-related differences were compared between the treatment group and the control group; effect sizes in the treatment group. Effect sizes were calculated using Cohen’s effect size formula (d), where an effect size of 0.20 is considered small, an effect of 0.50 medium, and an effect of 0.80 large. T1: Before training; T2: 5–6 weeks after training.
Training-related changes were compared between the treatment and control groups. This comparison revealed a significant treatment effect for two of four verbal working memory measures (digit span backward task, \( P < 0.05 \); word span backward task, \( P < 0.01 \)), a visuospatial working memory measure (Corsi span task, \( P < 0.01 \)), a central executive measure (time for completion of a Stroop task, \( P < 0.05 \)), and two literacy measures (accuracy and reaction time in a visual rhyming task, \( P < 0.05 \); one-minute tests of reading words, \( P < 0.01 \); Table 2).

With regard to the cognitive measures and the two literacy measures, the results showed a significant effect of training on both visuospatial working memory and reading achievement tasks (Table 2).

**Relationship between cognitive changes and reading skill changes following working memory training**

The relationship between training-related changes in literacy and cognitive measures in the treatment group were analyzed (Table 3).

| Item                      | Accuracy on visual rhyming task | Reaction time on visual rhyming task | One-minute tests of reading words |
|---------------------------|---------------------------------|-------------------------------------|----------------------------------|
| Digit span backward       | 0.42                            | 0.51                                | 0.47                             |
| Word span task backward   | 0.68                            | 0.49\(^{a}\)                        | 0.52                             |
| Corsi span task           | 0.56\(^{b}\)                    | 0.73\(^{b}\)                       | 0.59\(^{b}\)                     |
| Time for completion of a  | 0.48                            | 0.55                                | 0.72\(^{b}\)                     |
| Stroop task               |                                 |                                     |                                  |

\(^{a}\)\( P < 0.05 \), \(^{b}\)\( P < 0.01 \).

Progress on word span backward scores was positively correlated with progress on accuracy in a visual rhyming task. Progress on the Corsi span task was positively correlated with progress on literacy measures (accuracy and reaction time in a visual rhyming task and one-minute tests of reading words). The time for completion of a Stroop task was positively related to progress on one-minute tests of reading words. Finally, differences in training-related changes in literacy and cognitive measures were regressed to T1 in the treatment group. The results showed that training-related changes in the Corsi span task and accuracy and reaction time in a visual rhyming task and one-minute tests of reading words were inversely predicted by performance at T1 (Table 4).

In other words, children performing the worst at T1 improved the most at T2. None of the changes in other skills (T2–T1) regressed to T1 showed any significant difference.

**DISCUSSION**

This study is the first to examine working-memory training in Chinese children with developmental dyslexia. The aim was to investigate whether working-memory training improves dyslexic children’s working memory and reading skills. We found that intensive and adaptive computerized working-memory training gradually increased the amount of information that the subjects could keep in working memory. The results from the baseline and post-training tests in the treatment group were compared with the control group, which received a low-dose version of the training. This comparison showed that the training indeed enhanced the children’s working memory. Increased performance was seen in nontrained verbal working memory tasks, visuospatial working memory tasks, and central executive tasks, showing that the training effects generalized to other capacities. A significant training effect was also seen for both the visual rhyming task and reading fluency task (one-minute tests of reading words), and the effect size was substantial. Thus, it is possible to use working-memory training to improve cognitive function, which is an important deficiency in Chinese children with developmental dyslexia. However, future studies are required to determine the persistence of these effects.

Our experimental findings of significant effects of working-memory training on non-trained working memory tasks within the spatial, verbal, and central executive domains are consistent with previous studies of working-
memory training in children and adults. Additional interesting findings of the present study were the improvements on the digit span backward and word span backward task, which are consistent with transfer of the training\textsuperscript{11, 13, 16-17}. However, a significant training effect was not seen for either the digit span forward task or the word span forward task. Several studies have demonstrated training effects on non-trained tests of verbal working memory, with the dependent measure of the mean number of points on both the forward and backward condition\textsuperscript{13, 18}. Some studies showed that recalling digits or words in the inverse order of presentation is generally considered to involve both the phonological loop and the central executive\textsuperscript{19-20}, as the sequence spoken by the experimenter must be stored and reversed to produce the correct answer. However, another interpretation of the processes involved in these tasks has been proposed by Li et al\textsuperscript{21-22}, with a series of converging evidence suggesting that backward recall relies on visuospatial representation of the input material. In other words, recalling digits or words in the inverse order of presentation may use more processing resources of working memory than recall of forward presentations. As such, they are easier to individually assess. The results of this study demonstrate that comprehensive working-memory training was more effective for the digit span backward and word span backward task.

A significant training effect was also seen for both the visual rhyming task and reading fluency task. Correlation analyses of training-related changes between literacy measures and cognitive measures in the treatment group confirmed a positive relationship between improvements in reading skills and increased working memory capacity. These findings are in line with those of previous studies. For example, Shiran and colleagues\textsuperscript{15} stressed the importance of working-memory training for reading skills. It has been reported that 6 weeks of working-memory training in adult dyslexic readers increased literacy measures, such as phonology measures, silent reading times, and one-minute tests of reading words and pseudowords. Consequently, improvements in reading skills are probably related to the fact that reading skills depend on working memory. In other words, trained working memory tasks and reading skills rely on the same cortical areas. Some imaging investigations\textsuperscript{23-24} have revealed that the left dorsal lateral prefrontal cortex and premotor cortex, regions related to working memory, are relevant to Chinese reading.

As predicted, the weakest performers improved the most on the visuospatial working memory and central executive tasks and in their reading skills. This suggests that children performing at the lowest level may improve the most following training on visuospatial working memory tasks, central executive tasks, and reading skills. This supports the notion that the enlargement of working memory supply may contribute to reading effectiveness and focused reading. It is important to note that the effectiveness of the training was more pronounced among the lowest-level performers as they had more to gain in working memory and reading skills.

The present study indicates that training of working memory may be useful for children with development dyslexia. Our findings highlight the relationship between larger working memory capacities and better reading skills. Thus, this study has important practical implications in that children with Chinese development dyslexia may benefit from working-memory training. Because the use of behavioral measures to study working memory is limited, future research should investigate how effective remediation is associated with increased activation or normalization of brain functions in dyslexic children. It is clear that more research in this direction is warranted.

SUBJECTS AND METHODS

Design
A double-blind, paired designed study.

Time and setting
The training was conducted at one primary school in Wuhan, Hubei Province, China from May to June in 2011. Data collection and analysis were performed from January to September in 2011.

Subjects
The participants were third- or fifth-graders (aged 8–11 years) from one primary school in Wuhan, China. This primary school is located in an urban community of average-level socioeconomic status in Wuhan, China.

Diagnosis criteria
All the dyslexic children were diagnosed according to criterions defined by the International Statistical Classification of Diseases and Related Health Problems (Tenth Revision, ICD-10), issued by World Health Organization\textsuperscript{25}.

Inclusion criteria
Several inclusion criteria were used to ensure that all
participants: (1) Vocabulary test scores were at least 1.5 standard deviations lower than children in the same grade, as assessed by the Character Recognition Measure and Assessment Scale for Primary School Children[28]. (2) The score on the Dyslexia Checklist for Chinese Children was 2 standard deviations higher than the mean score[27]. (3) The children had normal nonverbal Raven intelligence quotients (intelligence quotient ≥ 85). (4) The children were physically healthy and had no history of neurological disease, head injuries, or psychiatric disorders. (5) Informed consent was obtained from each subject and their parents before initiating testing.

Methods

Procedure
During the week prior to the onset of training, the participants completed a set of assessments in working memory performance and reading achievement. Reading-related skills in the control group were measured within the same time interval as the treatment group. The format of the training was customized for each individual. All children completed two sessions of assessments within the same time intervals: (1) pre-test (T1), (2) post-test 5–6 weeks (T2).

The children in the treatment group engaged in training on a variety of working memory tasks using a computerized game environment. This training was conducted for approximately 40 minutes a day in the school over a period of 5 weeks. Children completed 100–150 trials every day. The time to complete each trial was reduced by 10% when the accuracy of the child in the treatment group increased by one fifth in each task. At the end of each day of exercises, children were allowed to choose a small tangible reinforcement from items such as sports or action figure cards, colored pencils, or a cartoon exercise book. Consistent with Torkel Klingberg’s experimental design[11], the control group was trained with a “placebo” or “low-dose” computer program, which was similar to the treatment program except its difficulty level was not interactively adjusted and daily training amounted to less than 10 minutes per day.

Training program
All training programs were written in the C programming language.

Visuospatial working memory task: The task involved the immediate serial recall of visuospatial information. Children were presented with six matrixes at 5 cm × 5 cm, with 3–5 colored squares in various positions (two matrixes for each of the three, four, or five square conditions). After the matrix had been removed, the child was asked to remember the positions of the colored squares and to point them out on an identical blank matrix[21, 28].

Visual verbal working memory tasks: For the training task, we used a similar paradigm to the one described by Miller et al[29]. The tasks in this paradigm are designed to reveal the nature of the working memory codes individuals rely on when asked to temporarily retain written words. Characters are selected from textbooks used in grades one to five. The average frequency of the characters was 17.64 per million, and the average number of strokes was 8.21. The participants were shown sequences of unrelated single target words, one after another on a computer screen. Immediately thereafter, they were asked to memorize a sequence of the first three target characters. Five Chinese characters were presented sequentially and displayed in a set order followed by a blank interstimuli presentation interval. The probe was three characters in the middle of the computer display. Only when both the orthography and order matched the three target characters was the subject supposed to press their index finger to indicate “yes” (A key of computer keyboard), otherwise, they were supposed to press their middle finger to indicate “no” (L key of computer keyboard). The tasks fell under a phonological condition designed to track reliance on a phonological memory strategy (Figure 1A), a visual condition designed to track reliance on a visual-perception-based memory strategy (Figure 1B), and a control condition (Figure 1C). In the phonological condition, the fourth characters resembled the target words phonologically, and were visually similar to those in the visual condition. The fifth characters were the distracter characters, and had no shared semantic, categorical, or linguistic features with the target words in any condition. Each condition was based upon 50 trials.

Central executive tasks: An inhibiting task is an often used version of the task used in this study[30]. Participants are required to give a fast left- or right-hand response to a central target arrow during presentation of congruent flanker (i.e., target arrow and flankers associated with the same response) or incongruent flanker (i.e., target arrow and flankers associated with different responses) arrows. No responses were required for non-target, octagon figures presented instead of the target. The flanker arrows were presented before the target arrow. The participants received 150 trials consisted of 50 congruent flankers, 50 incongruent flankers, and 50 octagon figures.
The study was a double-blind study where children, parents, and the psychologist administrating the baseline and post-training tests were blinded to the version of the computer program the children had practiced, and to the expected effects of the two versions. All training was completed in a quiet room in the school, in small groups of five children supervised by a training aide who was a paid research associate. Evaluation included nontrained working memory measures and reading achievement.

**Tests for the evaluation of the training**

The study was a double-blind study where children, parents, and the psychologist administrating the baseline and post-training tests were blinded to the version of the computer program the children had practiced, and to the expected effects of the two versions. All training was completed in a quiet room in the school, in small groups of five children supervised by a training aide who was a paid research associate. Evaluation included nontrained working memory measures and reading achievement.

**Working memory measures:**

1. **Verbal working memory:**
   a. **Digit span task**
      The digit span task is one of the tasks most often used to measure verbal working memory. It is part of the Wechsler Intelligence Scale for children, including the digit span forward task and the digit span backward task.
      In the first part, the digits are repeated in the same order as presented. In the second part, the child is asked to repeat the digits in the reverse order. The score corresponds to the maximum length of the item set recalled in the correct order of presentation.
   b. **Word span task**
      For the purpose of measuring children’s verbal working memory for words instead of numbers, a word span test was used[31-32]. It consisted of a series of frequently occurring Chinese words. Administration of the task and the scoring method were similar to those used for the Digit Span Subtest from the Wechsler Intelligence Scale for children.

2. **Visuospatial working memory:**
   **Corsi span task**
   This is a well-known task, very frequently used to measure short-term memory for spatial sequential information[11, 13, 33]. In addition, the task is considered to involve the encoding of visual stimuli, the retention of information over time, and response selection, prior to overt response execution[34]. From two up to nine lamps were presented sequentially in a 3 × 3 grid. These lamps were illuminated successively, and each child was asked to recall the correct order by clicking the appropriate location with the computer mouse. The number of Lamps in the sequence was successively increased until the subject missed two trials in a row. The score was the maximum number of lamps remembered.

3. **Central executive function:**
   **Stroop task**
   The Stroop task was used as a test of response central executive. Words describing colors were printed with ink in a color that was incongruent with the word. For example, the word “green” printed in yellow ink. The subjects were asked to name the ink color for each word. The time and accuracy for reading all 60 items was noted[35].

4. **Reading achievement:**
   a. **Phonological task**
      Phonological awareness was measured by the children’s accuracy and mean reaction time on correct responses on the visual rhyming task. The visual rhyming task we used was similar to the paradigm described by Grossi et al[36]. It contained 35 pairs of rhyming words and 35 pairs of nonrhyming words. Subjects needed to decide whether two sequentially presented written words rhymed or not. Each pair matching the average frequency of the characters and the average number of strokes selected from the textbooks used in grades one to five. The maximum score was 70.
    b. **One-minute tests of reading words**
      This task were similar to Li and colleague’s reading ability task[37]. 100 Chinese characters in the test were selected from textbooks that were used in primary schools for first to third graders, divided into 10 rows and 10 columns. Children were asked to read the characters aloud as quickly and accurately as possible within 1 min. Accuracy and reading fluency were scored.

**Statistical analysis**

Performance on non-trained working memory tasks and the two literacy measures is provided as mean ± SD.
Paired-samples t-tests were conducted to evaluate group differences. Effect sizes were calculated using Cohen’s effect size formula $d$, where an effect size of 0.20 is considered small, an effect of 0.50 medium, and an effect of 0.80 large. Furthermore, we conducted correlation analyses and regression analyses to determine the training effects. SPSS version 13.0 for windows software (SPSS, Chicago, IL, USA) was used for statistical analysis.

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