

Intermittent Hypoxia Does not Elicit Memory Impairment in Spinal Cord Injury Patients

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Abstract

There is a critical need for new therapeutic strategies to restore motor function in patients with spinal cord injuries (SCIs), without unwanted effects. Intermittent hypoxia (IH) induces plasticity in spared synaptic pathways to motor neurons below the level of injury, which can be harnessed to elicit motor recovery in incomplete SCI patients. However, there is conflicting evidence regarding the effects of IH on memory function. The aim of this study was to assess episodic verbal and visual memory function with the Complutense verbal learning test (TAVEC) and the Rey–Osterrieth Complex Figure Test (ROCF), respectively, before and after a 4-week protocol of repetitive IH combined with body weight-supported treadmill training (BWSTT) in incomplete ASIA C and D SCI subjects. Subjects received either IH (cycling 9%/21% FiO₂ every 1.5 min, 15 cycles per day) or continued normoxia (Nx, 21% FiO₂) combined with 45 min of BWSTT for 5 consecutive days, followed by 3 times per week IH and BWSTT for 3 additional weeks. ROCF Z scores between IH plus BWSTT and Nx plus BWSTT were not significantly different ($p = .43$). Compared with baseline, IH and BWSTT group showed a significantly greater ($p < .05$) verbal memory performance for immediate, short-term, and long-term recall; however, it was not different from Nx plus BWSTT group in all verbal memory components ($p > .05$). Our results suggest that a 4-week protocol of moderate IH does not elicit visual or verbal memory impairment. Thus, repetitive IH may be a safe therapeutic approach to incomplete spinal cord injury patients, without deleterious cognitive effects.

Keywords: Hypoxia; Learning and memory; Disability

Background

There is a critical need for new approaches to restore motor function in individuals with chronic spinal cord injuries (SCIs) and limited potential for recovery. Intermittent hypoxia (IH) has demonstrated to be a viable strategy to induce plasticity in spared synaptic pathways to motor neurons caudal to the level of injury (Devinney, Huxtable, Nichols, & Mitchell, 2013; Vinit, Lovett-Barr, & Mitchell, 2009), which can be harnessed to elicit motor recovery. Chronic, incomplete SCI patients, classified as Grade C or D on the American Spinal Cord Injury Association (ASIA) Impairment Scale (Marino et al., 2003), exposed to moderate and brief episodes of IH, increase their ability to voluntarily generate plantar flexion (Trumbower, Jayaraman, Mitchell, & Rymer, 2012), and increase their walking speed and resistance (Hayes et al., 2014).

Although IH may represent a potential therapy for incomplete SCI patients, there are reasonable concerns regarding the potential deleterious effects that IH has on memory function. Animal studies have demonstrated that protocols of chronic IH, similar to the hypoxia/re-oxygenation patterns observed in patients with obstructive sleep apnea (OSA), mimics memory deficits presented in subjects with OSA (Gozal, Daniel, & Dohanich, 2001; Veasey, 2009). However, the relative contributions of sleep fragmentation, daytime sleepiness, comorbid medical conditions, and IH exposure and magnitude are difficult to determine (Ferini-Strambi et al., 2003). For instance, cardiovascular risk factors, including diabetes, obesity, hypertension, and dyslipidemia, are independent risks factors for cognitive impairment in adults (Leritz, McGlinchey, Kellison, Rudolph, & Milberg, 2011; Panossian & Veasey, 2012; Prencipe et al., 2003).

Moreover, different protocols/paradigms of IH have the potential to elicit pathological or therapeutic effects at multiple systems, depending on the dose of IH (Champrod et al., 2013; Navarrete-Opazo & Mitchell, 2014; Yaffe et al., 2011). Healthy subjects exposed to moderate IH (3 min 13% FIO₂, 9 hr/day) for 28 consecutive nights showed no changes in sleepiness, encoding, attention, or working memory (Weiss et al., 2009). In contrast, exposure to severe IH (PETO₂ 45 Torr, every 1 min, for 6 hr) impairs working memory (Champrod et al., 2013). Severe protocols of IH elicit preferentially episodic memory impairment (Twigg et al., 2010; Wallace & Bucks, 2013). One study compared the memory deficit in patients with OSA versus healthy subjects. Those patients showed impaired verbal memory, whereas visual memory, working memory, and attention were unaffected (Twigg et al., 2010), which is supported by findings of a systematic review (Wallace & Bucks, 2013). Episodic verbal memory, including immediate and delayed recall, learning, and recognition, is impaired in OSA patients when compared with healthy controls (Wallace & Bucks, 2013).

In chronic SCI patients, a moderate protocol of IH (cyclical pattern of 9%/21% FIO₂ every 1.5 min, 15 cycles/day, for 5 days) does not impair cognitive function, using the Mini-Mental State Examination (MMSE) (Hayes et al., 2014). Although the MMSE is the most commonly administered psychometric screening assessment of cognitive functioning, its sensitivity to detect mild cognitive deficits is low (Benedict & Brandt, 1992; Tombaugh & McIntyre, 1992). Moreover, the effect of a longer (>5 days) exposure of IH on memory function in chronic SCI patients is unknown.

The purpose of this study is to assess episodic verbal and visual memory function before and after a 4-week protocol of moderate IH and body weight-supported treadmill training (BWSTT) in chronic incomplete ASIA C and D SCI patients.

Methods

Study Design

This was a multicenter, randomized, triple-blind, placebo-controlled parallel clinical trial conducted at Teletón Rehabilitation Institute, and included subjects from four hospitals in Santiago, Chile: “Instituto Teletón Santiago,” “Hospital Clínico Mutual de seguridad,” “Clínica Los Coihues,” and “Hospital del Trabajador.” Eligible subjects were randomized into two groups with restrictions based on ASIA and age to ensure balanced groups.

The study was conducted in accordance with the Declaration of Helsinki, and was approved by the institutional review boards of participating centers. All subjects provided written informed consent prior to study entry. This study reports the secondary outcomes of a greater trial testing the effectiveness of IH and BWSTT in walking speed and resistance in SCI patients (protocol registered at ClinicalTrials.gov NCT02441179).

The overall study and statistical analysis were reported according to CONSORT 2010 guidelines (Schulz, Altman, Moher, & Group, 2010) and SAMPL Guidelines (Lang & Altman, 2015), respectively.

Subjects

Men and women with spinal cord injuries were eligible for enrollment according to the following criteria—Inclusion Criteria: (1) subjects \geq 18 years old; (2) neurological level C5 or below, classified as ASIA grades C and D; (3) traumatic and non-traumatic, non-progressive lesions; (4) onset >6 months; (5) ability to ambulate with or without assistive devices; (6) ability to follow verbal or visual commands; and (7) signed informed consent. Exclusion Criteria: (1) orthopedic injuries that are unstable, (2) osteoporosis with high risk of pathological fracture, (3) cutaneous lesions and/or pressure ulcers, (4) joint contractures, (5) cardiopulmonary conditions, and (6) body weight exceeding 150 kg.

Intervention

Eligible subjects were randomly allocated into two groups through computer-generated random numbers with restrictions based on ASIA and age to ensure balanced groups. Half of the patients ($n = 18$) received IH plus BWSTT, whereas the other half ($n = 17$) received placebo (continued normoxia [Nx]) plus BWSTT. The physician administering the protocol of IH/placebo received the group assignment in sealed envelopes. Subjects, psychologists, and the external statistician were blind to group assignment, constituting a triple-blind study.

IH/placebo gas delivery. A gas mixing system was provided by tanks precalibrated and certified with the desirable concentration of gases (9% and 21% oxygen). The time of the intervals was automatically controlled by a programmable relay (Zelio Logic SR2B121BD) controlling the application of gas mixtures through solenoid valves. Subjects breathed the desirable concentration of humidified gases through a non-rebreathing mask attached to a tube connected to a flow meter located at the outlet of the solenoid

valves. The inspired oxygen concentration was monitored with an oxygen analyzer (Viamed AX300), and the blood oxygen saturation was monitored with a pulse oxymeter to assure that saturation does not drop below 80% during the hypoxic episodes. The protocol of placebo (continued Nx) used the same system, but only precalibrated normoxic (21%) air was delivered.

IH protocol. IH protocol consists of fifteen 90-s hypoxic episodes ($\text{FiO}_2 = 0.09$) interspersed with fifteen 90-s normoxic intervals ($\text{FiO}_2 = 0.21$) for a total time of 45 min. This protocol was repeated every day for five consecutive days and then three times per week for 3 weeks. Total time was 4 weeks.

Placebo protocol. Placebo protocol consists of continuous Nx ($\text{FiO}_2 = 0.21$) for 45 min for five consecutive days and then three times per week for 3 weeks. Total time was 4 weeks. The timing of solenoid valves was identical to the protocol of IH to avoid giving clues to the patients or the participant personal.

Body weight-supported treadmill training. After the protocol of IH/placebo, all subjects received training in BWSTT, starting at a speed of 0.6 km/hr for 45 min. The physical therapist manually corrected the patient's posture to assure an adequate gait, increasing the speed of treadmill progressively depending on the patient's progress and tolerance.

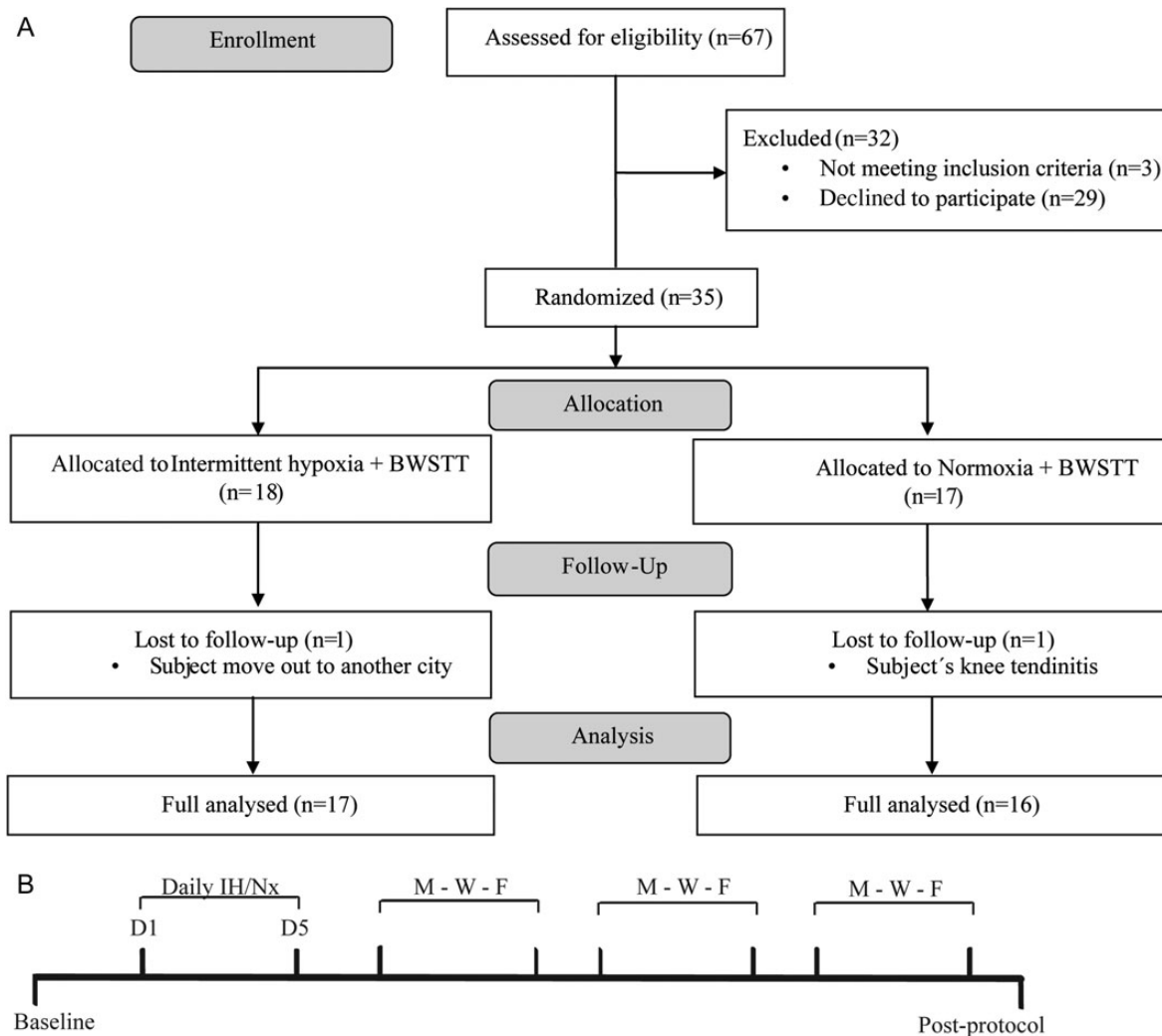


Fig. 1. (A) Flowchart showing recruitment, randomization, and final analysis from subjects allocated to two groups: (1) experimental group received IH plus BWSTT and (2) placebo group received Nx plus BWSTT. (B) Experimental design shows that subjects were exposed to IH (cycling 9%/21% FiO_2 every 1.5 min) or Nx (21% FiO_2) for five consecutive days followed by three times per week (Monday–Wednesday–Friday) exposure to IH/Nx, for three additional weeks. All subjects received BWSTT. Verbal and visual memory function was assessed with the TAVEC and ROCF, respectively, before (baseline) and after a 4-week protocol of IH/Nx.

In summary, two groups of patients received either IH or Nx (45 min), followed by BWSTT (45 min) for a total 90 min per day for five consecutive days and then three times per week for 3 weeks. Total time was 4 weeks (Fig. 1).

Outcome Measurements

The psychological assessments were conducted individually by two experienced psychologists. After sociodemographic data were collected, the same professional blindly administered the TAVEC and the Rey–Osterrieth Complex Figure Test (ROCF), before and after the IH/Nx plus BWSTT 4-week protocol. Each session lasted 1 h.

Spanish Complutense verbal learning test (TAVEC). The TAVEC is the Spanish version of the California Verbal Learning Test (Delis, Freeland, Kramer, & Kaplan, 1988) and is used for the assessment of episodic verbal memory (Benedet & Alejandro, 1998). The following verbal memory components were assessed: (1) total immediate recall, (2) short-term free recall, (3) long-term free recall, and (4) recognition.

The Rey–Osterrieth Complex Figure Test. The ROCF is a neuropsychological instrument used for the assessment of episodic visual memory (Meyers & Meyers, 1996; Rey, 1941). Subjects are presented with a complex line drawing and are asked to copy it freehand after 3 min (recognition). Once the first copy is complete, the figure and the subjects' copy are removed from view. Finally, 30 min later, subjects are asked to draw the figure from memory (recall). Subjects are not told beforehand that they will be asked to draw the figure 30 min later. In this study, we assessed the recall component of the test. Each subject's drawing was scored for the accurate reproduction and placement of 18 specific design elements according to the traditional guidelines developed by Taylor described in Spreen and Strauss (Taylor, 1998).

Statistical Analysis

Sample size. The memory outcomes reported here correspond to secondary outcomes of a greater trial testing the effectiveness of IH plus BWSTT in SCI patients. Thus, the sample size was obtained based on the 10-Meter Walk Test, the primary outcome (Navarrete-Opazo, Alcayaga, Sepulveda, Rojas, & Astudillo, unpublished results). Using a *t*-test for paired and independent samples (Dupont & Plummer, 1990), we calculated a sample size of 32 patients (16 subjects per group) for a parallel clinical trial with a 0.05 α error and 80% power.

Normality tests. Data from the memory tests were not normally distributed (Shapiro–Wilk $p < .05$). There was equality of variances for Groups 1 and 2 (Levene's test $p > .05$).

Psychological tests. Mann–Whitney nonparametric test was used to analyze differences between independent variables (IH vs. Nx). Wilcoxon test was used to compare visual and episodic memory function before and after a protocol of IH plus BWSTT or Nx plus BWSTT. Z-scores were obtained for verbal and visual memory with the following formula: $x - \mu/s$, where x is the direct score of the subject, μ the population mean score for that age range, and s is the population standard deviation. Data are presented as median (interquartile range). Age of subjects was presented as mean (*SD*).

Results

Subject's Eligibility

The subjects selected correspond to patients from four medical centers in Santiago, Chile: “Hospital Mutual de seguridad,” “Instituto Teletón,” “Clínica los Coihues,” and “Hospital del Trabajador.” A total of 67 subjects matched inclusion and exclusion criteria, based on the electronic database of each hospital. These patients were contacted by phone and invited to participate. Only 38 accepted the invitation and were assessed on site. Three subjects could not be included due to brain trauma history and cognitive deficits. Thus, 35 subjects were enrolled and randomly assigned to two groups: Group 1: IH plus BWSTT ($n = 18$), or Group 2: Nx plus BWSTT ($n = 17$). Out of 35 subjects, 33 completed the entire protocol and the memory assessments. One subject from the IH plus BWSTT group dropped the trial to move to another city and a second subject from the placebo group dropped the trial due to chronic knee tendinitis that prevented him from continuing with the training. Figure 1 shows the CONSORT (Consolidated Standards of Reporting Trials) diagram.

Characteristic of Subjects

Characteristics of the participants are shown in Table 1. Most of the subjects were men ($n = 31$). The mean age was 41 ($SD: 17$) and 42 ($SD: 17$) years for Groups 1 and 2, respectively. There was a balanced distribution of ASIA C and D subjects in Group 1 (6 ASIA C and 12 ASIA D) and Group 2 (7 ASIA C and 10 ASIA D). Most subjects ($n = 25$) had chronic SCI (>2 years since the injury) and completed secondary education ($n = 15$). None of the subjects had a diagnosis of mental illness or history of consumption of psychiatric medication and/or drug abuse.

Study Safety

The IH/Nx exposure and training program were well tolerated by all patients. No incidents or unwanted side effects were reported. There were no significant changes in blood pressure and heart rate between IH and Nx group ($p > .05$). The oxygen concentration during the hypoxic episodes was tightly controlled by certification of the gas mixture (Indura S.A., Chile), which ranged from 8.8% to 9.2% O₂. Additionally, a gas analyzer located in the output line was used to register the FiO₂ delivered to the subjects, which ranged from 8.7% to 9.4% during the hypoxic intervals. The pulse oxygen saturation dropped to 80%–83.5% during the hypoxia episodes and was maintained between 20.7% and 21.3% in the placebo (Nx) group.

Table 1. Baseline characteristics of randomized subjects ($n = 35$)

Subject	Intervention	Age (years)	Gender	ASIA grade	Level of education	Level of injury
T05	1	22	F	D	T	T3
T10	1	21	F	D	T	T1
T07	2	19	M	D	S	T6
T12	2	26	F	D	T	C6
T02	2	22	M	C	S	L1
T06	1	21	M	D	S	T3
T04	2	25	M	D	T	T9
T03	1	26	M	C	T	T12
T08	1	23	M	D	S	C7
T01	1	20	M	D	S	T9
M03	2	54	M	D	S	L4
M05	2	50	M	C	S	T8
M01	2	58	M	D	P	C5
M04	1	63	M	D	P	C6
M08	1	28	M	D	T	C4
M07	2	60	M	D	S	C5
M09	2	39	M	D	S	T4
M10	1	44	M	D	S	L1
M06	2	52	M	C	P	L3
M11	2	60	M	C	P	T12
M13	1	60	M	C	S	T12
C01	2	52	F	D	T	L3
M12	1	59	M	C	P	T12
M15	2	59	M	D	P	C6
C03	1	27	M	C	T	T6
C05	1	32	M	D	T	T10
T11	2	19	M	C	S	T6
C02	1	71	M	C	S	C6
M16	2	58	M	D	S	C7
M17	1	40	M	C	S	L1
M18	1	59	M	D	P	L3
C06	2	19	M	C	S	C5
C05	2	46	M	C	T	C6
A01	1	53	M	D	S	C6
A02	1	35	M	D	S	T9
Mean (<i>SD</i>)		41 ± 17				

Note: ASIA = American Spinal Cord Injury Association Impairment Scale; 1 = intermittent hypoxia plus body weight-supported treadmill training; 2 = air (normoxia) plus body weight-supported treadmill training; F = female, M = male; T = tertiary education; S = secondary education; P = primary education; *SD* = standard deviation.

Effect of IH on Episodic Visual Memory Function

For both groups, there were no differences in Z scores before or after the interventions. There were no statistically significant differences in median values between baseline and after the protocol of IH (0.48 [IQR: 0.97] vs. 0.19 [IQR: 1.33], $p = .063$), or after the protocol of Nx (0.31 [IQR: 0.80] vs. 0.16 [IQR: 0.91], $p = .094$) (Fig. 2).

Comparisons between interventions (Table 2) demonstrated that there was no statistically significant difference ($p = .43$) between IH plus BWSTT and Nx plus BWSTT, showing that the protocol of IH combined with BWSTT does not affect episodic visual memory function in incomplete ASIA C and D SCI subjects.

Effect of IH on Episodic Verbal Memory Function

There was a significant improvement in verbal memory performance after the protocol of IH plus BWSTT in the immediate recall ($p = .001$), short-term recall ($p = .021$), and long-term recall ($p = .008$) components (Fig. 3, Table 3), compared with

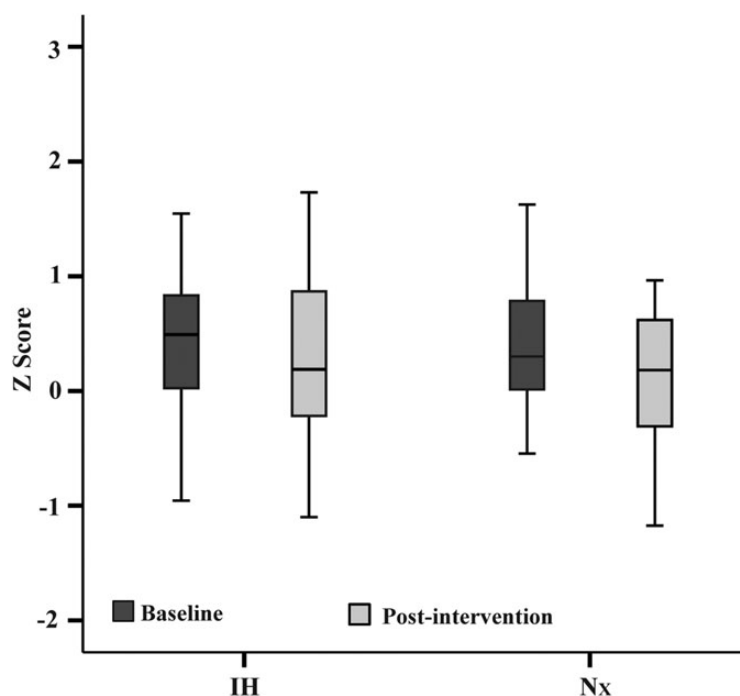


Fig. 2. Boxplots showing the median Z scores and interquartile range of the ROCF, before (dark gray boxes) and after (light gray boxes) a 4-week protocol of IH or Nx. Both groups received BWSTT. For both groups, there were no differences in Z scores before or after the interventions ($p > .05$). Comparisons between interventions demonstrated that there was no statistically significant difference between IH plus BWSTT and Nx plus BWSTT ($p = .43$).

Table 2. Episodic visual and verbal memory Z scores after interventions

Memory tests	IH + BWSTT		Nx + BWSTT		Mann–Whitney test <i>p</i> -Value
	Median	IQR	Median	IQR	
ROCF	0.19	1.33	0.16	0.91	.43
TAVEC					
TIR	0	1.5	−1	1	.55
STR	0	1	0	1.75	.97
LTR	0	1	0	1.75	.86
RCN	1	1	0.5	1	.77

Note: Data correspond to Z scores presented as median and interquartile range (IQR). ROCF = Rey–Osterrieth Complex Figure Test; TAVEC = Spanish Complutense verbal learning test; TIR = total immediate recall; STR = short-term free recall; LTR = long-term free recall; RCN = recognition; IH = intermittent hypoxia; Nx = normoxia; BWSTT = body weight-supported treadmill training.

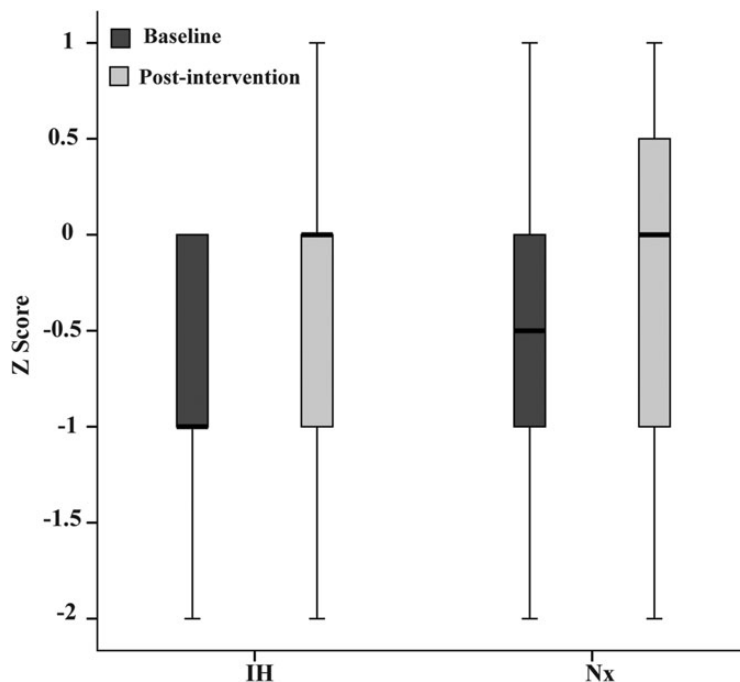


Fig. 3. Boxplots showing the median Z scores and interquartile range of the short-term recall component of the TAVEC, before (dark gray boxes) and after (light gray boxes) a 4-week protocol of IH or Nx. Both groups received BWSTT. There was a significant improvement in short-term recall verbal memory performance after IH plus BWSTT ($p = .021$). Group comparisons showed no statistical differences in Z scores between groups ($p > .05$).

Table 3. Episodic visual and verbal memory Z scores before and after interventions

Memory tests	IH + BWSTT		Wilcoxon test p	Nx + BWSTT		Wilcoxon test p
	Baseline	PI		Baseline	PI	
ROCF	0.48 (0.97)	0.19 (1.33)	.063	0.31 (0.8)	0.16 (0.91)	.094
TAVEC						
TIR	1 (0.5)	0 (1.5)	.001	-1 (1)	0 (1.7)	.002
STR	-1 (1)	0 (1)	.021	-0.5 (0)	0 (1.75)	.096
LTR	-1 (0)	0 (1)	.008	-0.5 (1)	0 (1.75)	.059
RCN	0 (1.5)	1 (1)	.273	0 (1.75)	0.5 (1)	.118

Note: Data correspond to Z scores presented as median and interquartile range (IQR). ROCF = Rey–Osterrieth Complex Figure Test; TAVEC = Spanish Complutense verbal learning; test; TIR = total immediate recall; STR = short-term free recall; LTR = long-term free recall; RCN = recognition; IH = intermittent hypoxia; Nx = normoxia; BWSTT = body weight-supported treadmill training; PI = post-intervention.

baseline values. Subjects receiving Nx plus BWSTT maintained their baseline Z scores for short-term and long-term recall; however, the immediate recall component significantly improved compared with baseline ($p = .002$).

Group comparisons showed no significant statistical differences in Z scores for all the components of TAVEC between IH plus BWSTT and Nx plus BWSTT groups ($p > .05$, Table 2).

Discussion

To our knowledge, this is the first study investigating the cognitive effects of a 4-week protocol of IH and BWSTT in incomplete ASIA C and D SCI patients. Specifically, we assessed episodic visual and verbal memory function with the ROCF and the Spanish Complutense Verbal Learning Test (TAVEC), respectively.

The main findings of this study were as follows: (1) repetitive IH does not affect episodic visual memory function; (2) repetitive IH improves episodic verbal memory function (compared with baseline) in the immediate, short-term, and long-term recall components; and (3) although there is a greater verbal performance after IH intervention, it was not statistically significantly different from placebo group (Nx plus BWSTT).

Moderate IH Does not Affect Episodic Memory Function

Our study did not find significant differences between a protocol of moderate IH plus BWSTT compared with Nx plus BWSTT on episodic verbal and visual memory performance in SCI patients. This is consistent with a previous study showing no cognitive impairment after 5 days of IH, using the MMSE (Hayes et al., 2014).

Animal studies have shown that exposure to IH during sleep can mimic OSA symptoms, including cognitive deficits (Gozal et al., 2001; Kheirandish, Gozal, Pequignot, Pequignot, & Row, 2005; Veasey, 2009), leading to the general conclusion that IH elicits memory impairment. However, most of the animal paradigms studying OSA use severe and/or high-frequent doses of IH (Row, Kheirandish, Cheng, Rowell, & Gozal, 2007; Titus et al., 2007; Xu et al., 2004). For instance, severe IH (cycling SaO₂ 65%–72%/>95% every 180 s, 12 hr/day, for 14 days) impairs spatial learning and working memory in mice (Dayyat, Zhang, Wang, Cheng, & Gozal, 2012). In contrast, mild IH (IH, 16.0% O₂, 4 hr/day for 4 weeks) enhances spatial learning and memory in postnatal developing mice (Zhang, Chen, Du, Chen, & Zhu, 2005).

Cognitive deficits are also observed in humans exposed to severe protocols of IH. For instance, exposure to severe IH (PETO₂ = 42 Torr, the equivalent of 76% arterial desaturation, every 1 min, for 6 hr) elicits spatial working memory impairment in healthy subjects (Champod et al., 2013). On the other hand, a moderate protocol of IH (cycling 13%/21% FIO₂ every 3 min, 9 hr a day) for 28 consecutive nights does not elicit attention or working memory in healthy subjects (Weiss et al., 2009). The differential effects of severe versus moderate protocols of IH are supported by studies showing the association between the severity of OSA and cognitive impairment (Kielb, Ancoli-Israel, Rebok, & Spira, 2012; Yaffe et al., 2011). Subjects with severe OSA (AHI ≥ 30) have a greater risk of cognitive deficits than those with moderate OSA (Kielb et al., 2012).

Cellular Mechanisms Underlying Severe Versus Moderate Protocols of IH

Different cellular mechanisms have been found in severe versus moderate paradigms of IH. In rats, exposure to severe doses of IH (cycling 5%–7%/21% FIO₂ every 90 s, more than 8 hr/day) elicits a deficit in spatial and working memory (Abdel-Wahab & Abd El-Aziz, 2012; Al-Qahtani, Abdel-Wahab, & Abd El-Aziz, 2014; Aubrecht, Weil, Magalang, & Nelson, 2013; Dayyat et al., 2012; Lam, Tipoe, So, & Fung, 2015), which has been associated with impaired synaptic plasticity (Wall, Corcoran, O'Halloran, & O'Connor, 2014), increased levels of reactive oxygen species (Al-Qahtani et al., 2014; Xu et al., 2004), neuronal apoptosis (Gozal et al., 2001; Row et al., 2007; Xu et al., 2004), increased inflammatory mediators (Carpagnano et al., 2002), increased hippocampal glutamate and thiobarbituric acid reactive substances (Al-Qahtani et al., 2014), and a decrease in antioxidant defenses (glutathione and glutathione peroxidase) (Al-Qahtani et al., 2014).

In contrast, a moderate dose of IH (cycling >9%/21% every 5 min, <8 hr/day) not only maintains memory function (Perry et al., 2008) but may also enhance spatial learning and memory performance (Lu et al., 2009; Zhang et al., 2005; Zhu et al., 2010). A moderate dose of IH does not elicit reactive gliosis or hippocampal cell death (Lovett-Barr et al., 2012), prevents ischemia-induced memory impairments (Rybnikova et al., 2005; Tsai, Yang, Chen, Chang, & Wang, 2008; Tsai, Yang, Sun, Liang, & Wang, 2013), and promotes neurogenesis (Pourie et al., 2006; Tsai et al., 2013; Zhu et al., 2010).

Cumulative evidence suggests that IH has markedly different cognitive effects depending on the frequency (cycles per day) and severity of inspired oxygen. Overall, protocols using <8% FiO₂ and frequent (48–2,400 episodes/day) cycles of hypoxia/re-oxygenation elicit cognitive impairment, whereas a less-frequent and milder dose of IH (≥8%) has beneficial effects (Navarrete-Opazo & Mitchell, 2014).

Study Limitations

Some points of the study have to be critically discussed as limitations, such as the small sample size, practice effect, and the protective effect of locomotor training.

Sample sizes in spinal cord injury studies are usually small, due to the difficulty of finding a homogenous population (Ginis & Hicks, 2005). In this study, our target population was incomplete SCI subjects (ASIA C and D) with strict inclusion/exclusion criteria, which prevented us from achieving a greater sample size. Further studies, with a greater statistical power, are needed in order to confirm our findings.

In this study, subjects receiving IH plus BWSTT exhibited a greater performance in the immediate recall, short-term recall, and long-term recall verbal memory tests, compared with baseline. Similarly, subjects receiving Nx plus BWSTT showed a greater performance in the immediate recall verbal memory component. The increased performance of both groups in the second assessment may be due to a practice effect. Because the time between assessments was 4 weeks, it is reasonable to think that subjects may have recalled the baseline test and performed better the second time.

Aerobic exercise may have exerted a protective effect, minimizing a negative impact of IH. Rats exposed to physical activity and IH (90 s of 10% FIO₂ alternating with 90 s of room air for 12 hr during the light phase) for 14 days showed no IH-induced spatial memory deficits (Gozal, Nair, & Goldbart, 2010). In elderly humans, strength-endurance training preceded by IH improves cognitive performance and sleep quality (Schega et al., 2013). Mechanisms explaining the beneficial effects of exercise on cognitive performance include: angiogenesis in brain (Kleim, Cooper, & VandenBerg, 2002) and cerebellum (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990), and increased brain-derived neurotrophic factor expression in hippocampus, enhancing synaptic plasticity and learning (Xie et al., 2010; Zhu et al., 2010). However, considering that both groups received physical training, we may reasonably conclude that all subjects experienced the positive effects of exercise.

Conclusions

Our results suggest that a 4-week protocol of moderate IH does not elicit visual or verbal memory impairment. Therefore, repetitive IH may be a safe therapeutic approach to incomplete spinal cord injury patients, without deleterious cognitive effects.

Cumulative evidence shows that IH has different effects, depending on the severity of the IH (Astorino, Harness, & White, 2015; Navarrete-Opazo & Mitchell, 2014). Severe protocols of IH elicit memory impairment, resembling cognitive deficits observed in most patients with OSA. In contrast, a moderate and low-frequent dose of IH may be harnessed as a therapeutic approach for multiple clinical disorders.

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Conflict of Interest

None declared.

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References

- Abdel-Wahab, B. A., & Abd El-Aziz, S. M. (2012). Ginkgo biloba protects against intermittent hypoxia-induced memory deficits and hippocampal DNA damage in rats. *Phytomedicine*, *19*, 444–450.
- Al-Qahtani, J. M., Abdel-Wahab, B. A., & Abd El-Aziz, S. M. (2014). Long-term moderate dose exogenous erythropoietin treatment protects from intermittent hypoxia-induced spatial learning deficits and hippocampal oxidative stress in young rats. *Neurochemical Research*, *39*, 161–171.
- Astorino, T. A., Harness, E. T., & White, A. C. (2015). Efficacy of acute intermittent hypoxia on physical function and health status in humans with spinal cord injury: A brief review. *Neural Plasticity*, *2015*, 409625.
- Aubrecht, T. G., Weil, Z. M., Magalang, U. J., & Nelson, R. J. (2013). Dim light at night interacts with intermittent hypoxia to alter cognitive and affective responses. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *305*, R78–R86.
- Benedet, M. J., & Alejandre, M. Á. (1998). *TAVEC: test de aprendizaje verbal España-Complutense: manual*. Madrid: TEA ediciones.
- Benedict, R. H., & Brandt, J. (1992). Limitation of the Mini-Mental State Examination for the detection of amnesia. *Journal of Geriatric Psychiatry and Neurology*, *5*, 233–237.
- Black, J. E., Isaacs, K. R., Anderson, B. J., Alcantara, A. A., & Greenough, W. T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 5568–5572.
- Carpagnano, G. E., Kharitonov, S. A., Resta, O., Foschino-Barbaro, M. P., Gramiccioni, E., & Barnes, P. J. (2002). Increased 8-isoprostane and interleukin-6 in breath condensate of obstructive sleep apnea patients. *CHEST Journal*, *122*, 1162–1167.
- Champod, A. S., Eskes, G. A., Foster, G. E., Hanly, P. J., Pialoux, V., Beaudin, A. E., et al. (2013). Effects of acute intermittent hypoxia on working memory in young healthy adults. *American Journal of Respiratory and Critical Care Medicine*, *187*, 1148–1150.
- Dayyat, E. A., Zhang, S. X., Wang, Y., Cheng, Z. J., & Gozal, D. (2012). Exogenous erythropoietin administration attenuates intermittent hypoxia-induced cognitive deficits in a murine model of sleep apnea. *BMC Neuroscience*, *13*, 77.
- Delis, D. C., Freeland, J., Kramer, J. H., & Kaplan, E. (1988). Integrating clinical assessment with cognitive neuroscience: Construct validation of the California Verbal Learning Test. *Journal of Consulting and Clinical Psychology*, *56*, 123–130.
- Devinney, M. J., Huxtable, A. G., Nichols, N. L., & Mitchell, G. S. (2013). Hypoxia-induced phrenic long-term facilitation: Emergent properties. *Annals of the New York Academy of Sciences*, *1279*, 143–153.
- Dupont, W. D., & Plummer, W. D., Jr. (1990). Power and sample size calculations. A review and computer program. *Controlled Clinical Trials*, *11*, 116–128.

- Ferini-Strambi, L., Baietto, C., Di Gioia, M. R., Castaldi, P., Castronovo, C., Zucconi, M., et al. (2003). Cognitive dysfunction in patients with obstructive sleep apnea (OSA): Partial reversibility after continuous positive airway pressure (CPAP). *Brain Research Bulletin*, *61*, 87–92.
- Ginis, K. A. M., & Hicks, A. L. (2005). Exercise research issues in the spinal cord injured population. *Exercise and Sport Sciences Reviews*, *33*, 49–53.
- Gozal, D., Daniel, J. M., & Dohanich, G. P. (2001). Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. *Journal of Neuroscience*, *21*, 2442–2450.
- Gozal, D., Nair, D., & Goldbart, A. D. (2010). Physical activity attenuates intermittent hypoxia-induced spatial learning deficits and oxidative stress. *American Journal of Respiratory and Critical Care Medicine*, *182*, 104–112.
- Hayes, H. B., Jayaraman, A., Herrmann, M., Mitchell, G. S., Rymer, W. Z., & Trumbower, R. D. (2014). Daily intermittent hypoxia enhances walking after chronic spinal cord injury: A randomized trial. *Neurology*, *82*, 104–113.
- Kheirandish, L., Gozal, D., Pequignot, J. M., Pequignot, J., & Row, B. W. (2005). Intermittent hypoxia during development induces long-term alterations in spatial working memory, monoamines, and dendritic branching in rat frontal cortex. *Pediatric Research*, *58*, 594–599.
- Kielb, S. A., Ancoli-Israel, S., Rebok, G. W., & Spira, A. P. (2012). Cognition in obstructive sleep apnea-hypopnea syndrome (OSAS): Current clinical knowledge and the impact of treatment. *Neuromolecular Medicine*, *14*, 180–193.
- Klein, J. A., Cooper, N. R., & VandenBerg, P. M. (2002). Exercise induces angiogenesis but does not alter movement representations within rat motor cortex. *Brain Research*, *934*, 1–6.
- Lam, C.-S., Tipoe, G. L., So, K.-F., & Fung, M.-L. (2015). Neuroprotective mechanism of Lycium barbarum polysaccharides against hippocampal-dependent spatial memory deficits in a rat model of obstructive sleep apnea. *PLoS One*, *10*, e0117990.
- Lang, T. A., & Altman, D. G. (2015). Basic statistical reporting for articles published in biomedical journals: The “Statistical Analyses and Methods in the Published Literature” or the SAMPL Guidelines. *International Journal of Nursing Studies*, *52*, 5–9.
- Leritz, E. C., McGlinchey, R. E., Kellison, I., Rudolph, J. L., & Milberg, W. P. (2011). Cardiovascular disease risk factors and cognition in the elderly. *Current Cardiovascular Risk Reports*, *5*, 407–412.
- Lovett-Barr, M. R., Satriotomo, I., Muir, G. D., Wilkerson, J. E., Hoffman, M. S., Vinit, S., et al. (2012). Repetitive intermittent hypoxia induces respiratory and somatic motor recovery after chronic cervical spinal injury. *Journal of Neuroscience*, *32*, 3591–3600.
- Lu, X. J., Chen, X. Q., Weng, J., Zhang, H. Y., Pak, D. T., Luo, J. H., et al. (2009). Hippocampal spine-associated Rap-specific GTPase-activating protein induces enhancement of learning and memory in postnatally hypoxia-exposed mice. *Neuroscience*, *162*, 404–414.
- Marino, R. J., Barros, T., Biering-Sorensen, F., Burns, S. P., Donovan, W. H., Graves, D. E., et al. (2003). International standards for neurological classification of spinal cord injury. *Journal of Spinal Cord Medicine*, *26*(Suppl. 1), S50–S56.
- Meyers, J. E., & Meyers, K. R. (1996). *Rey Complex Figure Test and Recognition Trial: Supplemental norms for children and adults*. Psychological Assessment Resources.
- Navarrete-Opazo, A., & Mitchell, G. S. (2014). Therapeutic potential of intermittent hypoxia: A matter of dose. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *307*, R1181–R1197.
- Navarrete-Opazo, A., Alcayaga, J., Sepulveda, O., Rojas, E., & Astudillo, C. Repetitive intermittent hypoxia and locomotor training enhances walking function in incomplete spinal cord injury subjects. Unpublished.
- Panosian, L. A., & Veasey, S. C. (2012). Daytime sleepiness in obesity: Mechanisms beyond obstructive sleep apnea—a review. *Sleep*, *35*, 605–615.
- Perry, J. C., D’Almeida, V., Lima, M. M., Godoi, F. R., Vital, M. A. B., Oliveira, M. G. M., et al. (2008). Intermittent hypoxia and sleep restriction: Motor, cognitive and neurochemical alterations in rats. *Behavioural Brain Research*, *189*, 373–380.
- Pourie, G., Blaise, S., Trabalon, M., Nedelec, E., Gueant, J. L., & Daval, J. L. (2006). Mild, non-lesioning transient hypoxia in the newborn rat induces delayed brain neurogenesis associated with improved memory scores. *Neuroscience*, *140*, 1369–1379.
- Prencipe, M., Santini, M., Casini, A. R., Pezzella, F. R., Scaldaferrri, N., & Culasso, F. (2003). Prevalence of non-dementing cognitive disturbances and their association with vascular risk factors in an elderly population. *Journal of Neurology*, *250*, 907–912.
- Rey, A. (1941). L’examen psychologique dans les cas d’encéphalopathie traumatique. (Les problèmes.). *Archives de Psychologie*, *28*, 215–285.
- Row, B. W., Kheirandish, L., Cheng, Y., Rowell, P. P., & Gozal, D. (2007). Impaired spatial working memory and altered choline acetyltransferase (CHAT) immunoreactivity and nicotinic receptor binding in rats exposed to intermittent hypoxia during sleep. *Behavioural Brain Research*, *177*, 308–314.
- Rybnikova, E., Vataeva, L., Tyulkova, E., Gluschenko, T., Otellin, V., Peltto-Huikko, M., et al. (2005). Mild hypoxia preconditioning prevents impairment of passive avoidance learning and suppression of brain NGFI-A expression induced by severe hypoxia. *Behavioural Brain Research*, *160*, 107–114.
- Schega, L., Peter, B., Torpel, A., Mutschler, H., Isermann, B., & Hamacher, D. (2013). Effects of intermittent hypoxia on cognitive performance and quality of life in elderly adults: A pilot study. *Gerontology*, *59*, 316–323.
- Schulz, K. F., Altman, D. G., Moher, D., & Group, C. (2010). CONSORT 2010 statement: Updated guidelines for reporting parallel group randomized trials. *Obstetrics & Gynecology*, *115*, 1063–1070.
- Taylor, L. (1998). Scoring criteria for the Rey-Osterrieth complex figure test. *A Compendium of Neuropsychological Tests. Administration, Norms, and Commentary* (pp. 350–351). New York, NY: Oxford University Press.
- Titus, A. D., Shankaranarayana Rao, B. S., Harsha, H. N., Ramkumar, K., Srikumar, B. N., Singh, S. B., et al. (2007). Hypobaric hypoxia-induced dendritic atrophy of hippocampal neurons is associated with cognitive impairment in adult rats. *Neuroscience*, *145*, 265–278.
- Tombaugh, T. N., & McIntyre, N. J. (1992). The mini-mental state examination: A comprehensive review. *Journal of the American Geriatrics Society*, *40*, 922–935.
- Trumbower, R. D., Jayaraman, A., Mitchell, G. S., & Rymer, W. Z. (2012). Exposure to acute intermittent hypoxia augments somatic motor function in humans with incomplete spinal cord injury. *Neurorehabilitation and Neural Repair*, *26*, 163–172.
- Tsai, Y. W., Yang, Y. R., Chen, G. H., Chang, H. C., & Wang, R. Y. (2008). The time window of intermittent hypoxia intervention after middle cerebral artery occlusion. *Chinese Journal of Physiology*, *51*, 324–328.
- Tsai, Y. W., Yang, Y. R., Sun, S. H., Liang, K. C., & Wang, R. Y. (2013). Post ischemia intermittent hypoxia induces hippocampal neurogenesis and synaptic alterations and alleviates long-term memory impairment. *Journal of Cerebral Blood Flow & Metabolism*, *33*, 764–773.
- Twigg, G. L., Papaioannou, I., Jackson, M., Ghiassi, R., Shaikh, Z., Jaye, J., et al. (2010). Obstructive sleep apnea syndrome is associated with deficits in verbal but not visual memory. *American Journal of Respiratory and Critical Care Medicine*, *182*, 98–103.
- Veasey, S. (2009). Insight from animal models into the cognitive consequences of adult sleep-disordered breathing. *ILAR Journal*, *50*, 307–311.

- Vinit, S., Lovett-Barr, M. R., & Mitchell, G. S. (2009). Intermittent hypoxia induces functional recovery following cervical spinal injury. *Respiratory Physiology & Neurobiology*, *169*, 210–217.
- Wall, A. M., Corcoran, A. E., O'Halloran, K. D., & O'Connor, J. J. (2014). Effects of prolyl-hydroxylase inhibition and chronic intermittent hypoxia on synaptic transmission and plasticity in the rat CA1 and dentate gyrus. *Neurobiology of Disease*, *62*, 8–17.
- Wallace, A., & Bucks, R. S. (2013). Memory and obstructive sleep apnea: A meta-analysis. *Sleep*, *36*, 203–220.
- Weiss, M. D., Tamisier, R., Boucher, J., Lynch, M., Gilmartin, G., Weiss, J. W., et al. (2009). A pilot study of sleep, cognition, and respiration under 4 weeks of intermittent nocturnal hypoxia in adult humans. *Sleep Medicine*, *10*, 739–745.
- Xie, H., Leung, K. L., Chen, L., Chan, Y. S., Ng, P. C., Fok, T. F., et al. (2010). Brain-derived neurotrophic factor rescues and prevents chronic intermittent hypoxia-induced impairment of hippocampal long-term synaptic plasticity. *Neurobiology of Disease*, *40*, 155–162.
- Xu, W., Chi, L., Row, B. W., Xu, R., Ke, Y., Xu, B., et al. (2004). Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. *Neuroscience*, *126*, 313–323.
- Yaffe, K., Laffan, A. M., Harrison, S. L., Redline, S., Spira, A. P., Ensrud, K. E., et al. (2011). Sleep-disordered breathing, hypoxia, and risk of mild cognitive impairment and dementia in older women. *Journal of the American Medical Association*, *306*, 613–619.
- Zhang, J. X., Chen, X. Q., Du, J. Z., Chen, Q. M., & Zhu, C. Y. (2005). Neonatal exposure to intermittent hypoxia enhances mice performance in water maze and 8-arm radial maze tasks. *Journal of Neurobiology*, *65*, 72–84.
- Zhu, X. H., Yan, H. C., Zhang, J., Qu, H. D., Qiu, X. S., Chen, L., et al. (2010). Intermittent hypoxia promotes hippocampal neurogenesis and produces antidepressant-like effects in adult rats. *Journal of Neuroscience*, *30*, 12653–12663.