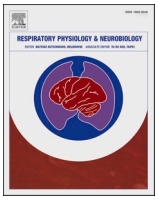
Respiratory Physiology & Neurobiology 300 (2022) 103885

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol



Epidural electrical spinal cord stimulation of the thoracic segments (T2-T5) facilitates respiratory function in patients with complete spinal cord injury

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ARTICLE INFO

Keywords:
Spinal Cord Epidural Electrical Stimulation (EES)
Phrenic Nerve Stimulation (PNS)
Diaphragm Electromyography (DEMG)
Inspired Volume (IV)
Continuous Positive Airway Pressure (CPAP)

ABSTRACT

Introduction: Patients with high cervical Spinal Cord Injury (SCI) usually require mechanical ventilation support. Phrenic Nerve Stimulation (PNS) both direct and indirect is the main alternative for these patients to wean off ventilator although PNS has several limitations and phrenic nerve could be also damaged after cervical spinal cord injury.
Objective: In this study, we assessed if the spinal cord Epidural Electrical Stimulation (EES) at the segments T2-T5, related to intercostal muscles, can facilitate respiratory function and particularly inspired tidal volume during mechanic ventilation.
Methods: Two patients with a high cervical injury were selected for this study with ethical committee permission and under review board supervision. A phrenic nerve conduction study with diaphragm electromyography (DEMG) was performed before and after trial of EES.
Results: Results demonstrate that EES at T2-T5 substantially increase the inspired volume. The results of this study also demonstrate that EES at spinal segments T2-T5 can bring patients dependent from mechanical ventilation to pressure support (on CPAP), preventing Baro-trauma and other complications related to mechanical ventilation.
Conclusion: These findings suggest that tested approach applied alone or in combination with the phrenic nerve stimulation could help to reduce time on mechanical ventilation and related complications.

1. Introduction

Cervical Spinal Cord Injury (SCI) is commonly related to respiratory complications and particularly patients with high cervical injury require continuous artificial ventilation, associated with multiple side effects, like lungs' infection and structural damage (Acker et al., 1987). Available alternatives include chronic implantation of the Phrenic Nerve Stimulation (PNS) (Acker et al., 1987), respiratory muscles stimulation (Adler et al., 2009), and spinal cord Epidural Electrical Stimulation (EES) (Agostoni et al., 1964). Phrenic nerve stimulation demonstrated efficient control over respiration in patients who require artificial ventilation support, secondary to high cervical injury (Brown et al., 2006) and secondary to brain injury, when phrenic nerve remains functional and implantation of stimulator can be performed (Adler et al., 2009). In patients with high cervical injury the functionality of phrenic nerve can be compromised (Agostoni et al., 1964) and phrenic nerve stimulation cannot be offered (Brown et al., 2006). Each hemi-diaphragm is innervated by its ipsilateral phrenic nerve, derived primarily from the C3 through C5 lower motor neurons (LMNs), most of which reside within the C4 spinal segment (Carter, 1989). Two different types of SCI can cause diaphragmatic paralysis and complete respiratory failure: injuries at the mid-cervical region at C2-C4, which directly damage phrenic LMNs, and lesion rostral to C3 level, which spare the phrenic LMNs, but cause damage of descending bulbo-spinal upper

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https://doi.org/10.1016/j.resp.2022.103885
Received 2 August 2021; Received in revised form 26 February 2022; Accepted 5 March 2022
Available online 8 March 2022

motor neurons (UMNs) pathways. It is important to note that large number of individuals with thoracic lesions or ventilator-independent cervical lesions may nonetheless also experience some degree of respiratory insufficiency (Decima et al., 1969). For example, a recent survey of patients admitted at SCI-specialized rehabilitation centers in the Netherlands reported that 30.9% patients had impaired forced Vital Capacity (VC) and 29.0% reported experiencing dyspnea during activity, while 18.4% experienced significant dyspnea even at rest (Decima et al., 1967). The accessory muscles involved in inspiration include the external intercostal muscles (T1-T11), the sternocleidomastoid muscles (cranial nerve XI), and the scalene muscles (C4-C6) (DiMarco et al., 1989). The intercostal muscles contribute to deep inhalation and critical for preventing or relieving atelectasis (DiMarco et al., 1989). Several works demonstrated that intercostal stimulation in animal model could help to restore breathing pattern (DiMarco et al., 1989, 1997b, 2004). This study was designed to evaluate the potential of EES applied at thoracic segments related to intercostal muscles (T2-T5) to facilitate self-breathing in patients with ventilator-dependent quadriplegia for whom the phrenic nerve pacing was not possible due to phrenic nerve and/or motor neurons damage. Two subjects with C2-C4 cervical injury were selected for this study.

2. Methods

2.1. Study subjects

Two trauma-induced ventilator-dependent subjects with quadriplegia were enrolled in this study. Both subjects **(S1 and S2)** were in stable condition before and after enrollment. Based on inclusion criteria, only highly motivated subjects with sufficient family and/or nursing support were selected for this study. Furthermore none of them suffered from primary pulmonary, cardiac, or brain disease. Both subjects had significant injury to the phrenic nerves and/or phrenic motor neurons that precluded the possibility of successful phrenic nerve pacing. The study was approved by the Institution of Brain and Spine hospitals Review Boards of the Medical Center (protocol number IR-2021–21-IPD-01–02). The informed consent was obtained before enrollment. Clinical characteristics of both subjects are shown in Table 1. The interval between the time after injury and entry in the study ranged from 15 days to 1 month. Both subjects demonstrated complete SCI with no motor or sensory functions below the injury level (AIS-A). The spontaneous vital capacity (VC) (Table 1) was markedly reduced in both subjects and was consistent with high cervical SCI. Based on initial evaluation both subjects required mechanical ventilation. The baseline arterial blood gas values and ventilator setting are presented in Table 2 (We have calculated tidal volume 6 ml/kg body weight for our subjects).

2.2. Electrode placement

Each subject underwent a T-7 or T-8 hemi-laminectomy procedure to implant the Medtronic spinal cord electrode (3877–60 Octad standard lead, LZ STD EAME, Medtronic, Inc, Minneapolis, USA) on the dorsal epidural surface of the upper thoracic (T1-T5) spinal segment (Fig. 1A). Previous studies indicate that optimal inspired volume with electrical spinal cord stimulation was achieved with stimulation at T2 spinal level. Accordingly, we attempted to position the center of the electrode at the midline over T2 segment (DiMarco et al., 1997b). With this initial procedure, the electrode wires were externalized to allow subsequent electrical stimulation of each lead. The external stimulator (external neuro stimulation – ENS - Programmer 37022, Medtronic, Inc, Minneapolis, USA) was used to deliver a biphasic stimulus and provide a pulse width-modulated impulse. During the initial period (first 4–5 days) electrical stimulation was applied at frequency of 40 Hz, pulse width of 210 μs and with the amplitude up to 3.5 V. Low settings during this period allowed to settle the edema and inflammation at the electrode site and healing of the wounds. Both subjects were subsequently evaluated and observed in the intensive care unit at the Medical Center to allow monitoring of physiological variables, while the effects of electrical stimulation were documented. In each instance, the stimulation with the lead close to T2-T4 spinal segments resulted in increase of inspired volume and negative inspiratory pressure (NIP).

Table 2
Ventilatory characteristics of quadriplegic patients (VT: tidal volume; RR: respiratory rate).

Subjects	Arterial blood gas volumes on mechanical ventilation				Ventilator Settings	
	pH	PCO_2 (mmHg)	PO_2 (mmHg)	O_2 saturation (%)	VT (ml/Kg)	RR (bpm)
S1	7.36	35	90	95	516 (6 ml/Kg)	10
S2	7.44	33	89	97	372 (6 ml/Kg)	10

2.3. Implantable pulse generator placement

A subsequent surgical procedure was performed for S1 at 7 days and for S2 at 10 days after the initial procedure. Two leads were connected to Medtronic pulse generator (INS 97702 prime advanced MRI, Medtronic, Inc. Minneapolis, USA). The pulse generator was implanted subcutaneously over the anterior rib cage to optimize the antenna placement during intercostal muscle stimulation (Fig. 1B).

3. Muscle reconditioning and diaphragm electromyography (DEMG)

Approximately 2 weeks after the second surgical procedure, both subjects started a reconditioning program. As the intercostal muscles had undergone significant atrophy and required a period of gradually increased muscle activation to restore strength and endurance, both patients initially underwent intercostal muscle pacing for 5 min/h, 4hrs/d with the help of implanted spinal cord stimulator and electrodes (T2-T5). The intensity and time of stimulation were gradually increased as tolerated. Stimulation frequency was maintained at 20 Hz as low-frequency stimulation expected to increase the fatigue-resistant activity of the type I fibers (DiMarco et al., 2009a; DiMarco and Kowalski, 2013b). For S1, who could not tolerate time-off mechanical ventilation support, with insufficient inspired volume production, EES was not efficient to maintain adequate ventilation and was performed in conjunction with mechanical ventilation. This was accomplished by setting of the mechanical ventilator in the assisted mode (DiMarco et al., 2004, 2009a, 2009b; DiMarco and Kowalski, 2011, 2013b). Diaphragmatic ultrasonography provided direct visualization of the diaphragm, qualitative evaluation of diaphragmatic excursion and diaphragm motion with M-mode ultrasonography. Patients were initially examined

Table 1
Clinical data of the quadriplegic patients.

Subjects	Sex & Age	Weight (Kg) & Height (cm)	Cause of the injury	Level of the injury	Elapsed time since injury	Spontaneous VC (ml)	Self-breaths/min (bpm)
S1	M (30 years)	86 kg & 180.34 cm	Driving accident	C2-C3	1 month	0 ml	0
S2	F (21 years)	62 kg & 165.10 cm	Driving accident	C3-C4	15 days	70–80 ml	2–3

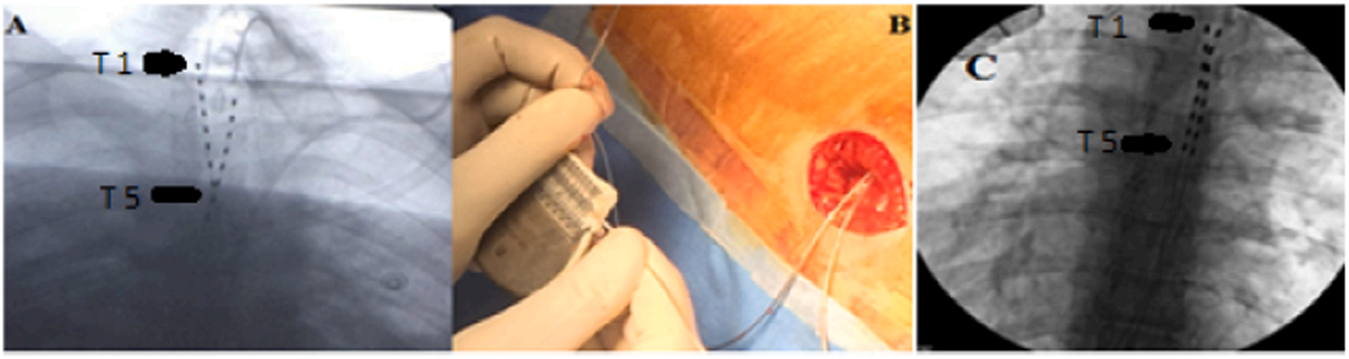

Fig. 1. (A). Lead placement on T-1 to T-5 region (intercostal location) for S1. (B). Implantable pulse generator fixation post-trial stimulation for both S1 and S2. (C). Lead placement on T1-T5 region for S2.

with and without mechanically assisted ventilations. For this the ventilator was temporarily disconnected for 12–15 s when the patient underwent PNS.

3.1. *Phrenic nerve conduction study before SCS at the intercostal region (T2-T5)*

All patients underwent a phrenic nerve conduction study before EES started to assess the phrenic nerve and its potential pacing, performed with bipolar surface stimulation electrodes with a standard recording montage. Stimulation right above the clavicle, between the sternal and clavicular heads of the sternocleidomastoid muscles, elicited responses at the lowest stimulation strength, without concomitant brachial plexus stimulation (DiMarco and Kowalski, 2009, 2011). A bipolar stimulating electrode (16893; Medelec, Old Woking, UK), two disposable self-adhesive disk recording electrodes (Viasys Healthcare, Madison, Wisconsin), and an EMG system (Key point; Medtronic Functional Diagnostics, Skovlunde, Denmark) with standard settings (filters 2 Hz to 10 kHz) were used (DiMarco and Kowalski, 2010). For compound muscle action potential (CMAP) recording the active electrode (G1) was fixed 5 cm above the xiphoid process and the reference electrode (G2) 16 cm from G1, on the chest margin ipsilateral to the stimulated phrenic nerve. Electrical stimulation was carried out with rectangular pulses of 0.1 ms duration. Measurements were made separately during normal inspiration and expiration with ultrasound control of the diaphragm condition and thickness of the effected diaphragm.

3.2. *Measurement of inspired volume*

The respiratory volumes were measured before and after EES, while subjects were on ventilation. The ventilation of Dräger equipment can be divided into three modes: volume-controlled, pressure-controlled, and spontaneous/assisted modes. Tidal Volume (VT) was assessed in all three modes. Post-stimulation assessment was performed with different frequencies, i.e. 20 Hz, 30 Hz, or up to 100 Hz for a week with the following comparison of VTs. The pulse width was adjusted (210 μs–300 μs) along with the EES amplitude (2–10 V) weekly and based on VT capacity in each subject.

3.3. *Evaluation of breathing per minute on CPAP mode*

After 10 weeks the capacity of each subject to maintain inspired volume and breathing frequencies was evaluated. For this, each subject was transferred to CPAP mode, initially for 2–3 min and, then, with gradually increased time on CPAP on daily basis with measuring the respiratory volumes and frequency of breathing. At that point we also measured the capacity to sustain the breathing with proper volumes along with control of other autonomic functions (like blood pressure, heart rate, and oxygen saturation). All observations and analyses were performed in the intensive care unit under supervision of interventionist.

4. Results

In all cases the onset of EES was visible with a palpable contraction of the upper trunk musculature and sometimes contraction of the upper arm and mild flexion of the hands. Contraction of the pectoral muscles and intercostal muscles of the upper four-five spaces was easily palpable. EMG was used to measure intercostal muscles and sternocleidomastoid muscles activity with and without stimulation. Initially, a small degree of asymmetry in movements was observed between the right and left sides of the body in S1, with some degree of inward abdominal movement. During the following 4 weeks of EES (frequency 40 Hz, pulse width 210μs, and amplitude 4.5 V, 12 h On and 12 h OFF) the asymmetric muscular contractions gradually decreased in both subjects. In addition, the degree of motion became less pronounced as the study progressed. S2 reported mild residual sensation in his right upper extremity post electrical stimulation as a buzzing sensation localized in his right arm. While this feeling was uncomfortable initially, sensation decreased and wasn't unpleasant after the first weeks of stimulation. S1 did not report any sensation related to the stimulation.

4.1. *Phrenic nerve conduction and diaphragm electromyography*

Reproducible phrenic nerve CMAPs were recorded in both subjects. The optimal position of the stimulating electrodes to evoke CMAP varied slightly between both subjects. Thus the stimulation between the sternal and clavicular heads of the Sternocleidomastoid muscle (SCM) just above the clavicle was mild at high stimulation intensities. The bipolar hand-held surface electrode was firmly pressed at the point between the SCM heads. To improve the stimulation, particularly on the left side, the electrode was moved laterally to the posterior border of the SCM, although, without significant difference. Another attempt with electrode placement at the junction of internal jugular vein and subclavian vein, where they form the brachiocephalic vein, did not improve response either. Diaphragmatic ultrasonography was performed in supine position. A multi frequency 4-MHz vector transducer was used in the longitudinal semi coronal plane, using a subcostal or low intercostal approach extending from the midaxillary to the midclavicular lines. The right diaphragm was visualized through the window of the liver and the left diaphragm was visualized though the window of the spleen. The ultrasonography recordings presented on Fig. 2(A & B).

4.2. *Inspired volume analysis vs. post stimulation at different week intervals*

Initial maximum inspired volumes and Negative Inspiratory Pressures (NIPs) during the first two weeks after implantation of the

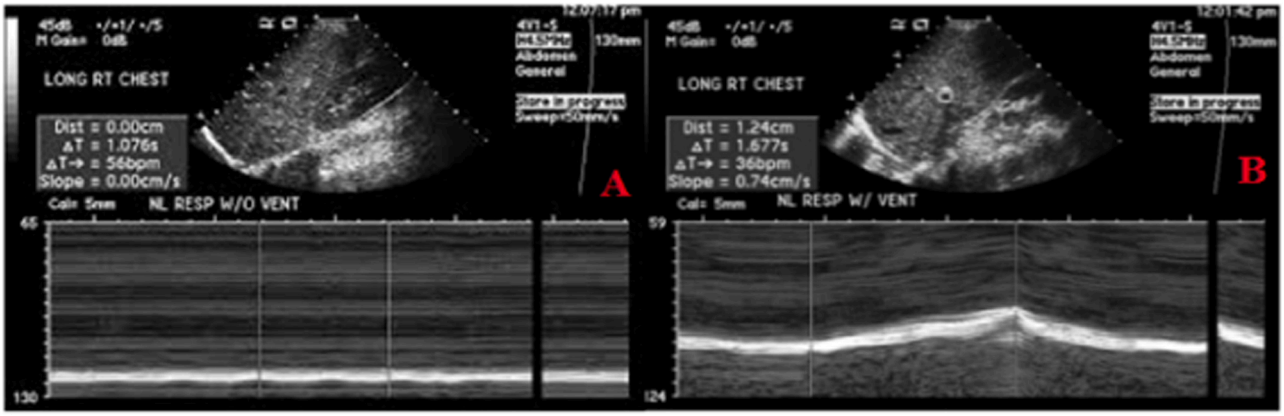

Fig. 2. (A). M-mode diaphragm ultrasonography during a breathing trial without mechanical ventilation in a subject with no spontaneous respiration. M-mode diaphragm ultrasonography with pacer amplitude at 1.0, 1.5, and 2.0 with this diaphragm excursion was zero. Note the flat white line indicating no movement of the diaphragm. (B). M-mode diaphragm ultrasonography during a trial with mechanical ventilation in a subject with spontaneous respirations. M-mode diaphragm ultrasonography with pacer amplitude at 1.0, 1.5 and 2.0 with the diaphragm excursion 0.74 cm, 1.10 cm, and 1.24 cm correspondingly. Note the white line indicating movement of the diaphragm.

electrode ranged between 150 and 180 ml and negative 1–3 cm H_2O, respectively. Trans-diaphragmatic pressure was zero during stimulation. The maximum inspired volumes, resulting from activation of intercostal muscles with EES at T2-5 segments at the different time points across this study presented on Fig. 3A. Inspiratory time (duration of pulse train) was maintained relatively constant at approximately 1.6 s. In S2, the inspired volumes increased progressively during the reconditioning period. As inspiratory time was relatively constant, an increase in inspired volume was achieved by a progressive increase in peak inspiratory flow rate. Maximum inspired volume achieved in S2 was 446 ml and maximum NIP was 10.0 cm H_2O. In S1 with EES initial maximum inspired volumes and negative inspiratory pressures (NIPs) during the first two weeks after implantation were ranged between 70 and 80 ml and negative 1 cm H_2O, respectively. Trans-diaphragmatic pressure was

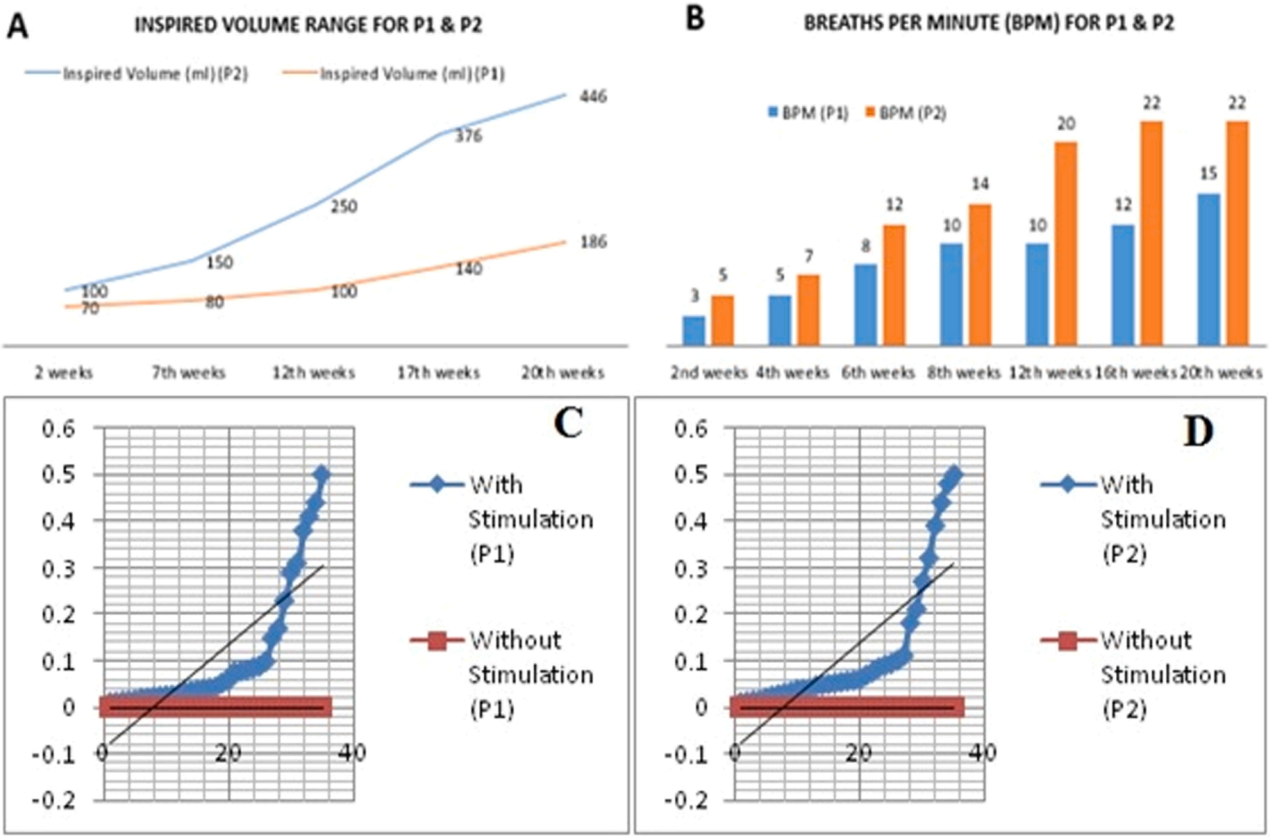

Fig. 3. (A). S1 and S2 maximum inspired volumes with EES during different time intervals across the study (2–20 weeks). As a result of gradually increased pacing duration, progressive increments in inspired volume were observed. (B). S1 post stimulation on CPAP system with self-breathing frequency per minute (bpm) 15–16 breathes in NIP 1 cm H_2O support, and S2, post stimulation on complete OFF ventilation system maintained around 40–45 min self-breath frequency around 20–22 breaths. (C & D). Diaphragm EMG calculative values with and/or without stimulation for S1 and S2 (Here Y axis refers the positive tidal volume scales ml/kg, blue lines showed post stimulation patients tidal volume started improving as 0.1 equal to 10 ml/kg tidal volumes and X axis refers the timeframe in weeks).

also zero during EES. Maximum inspired volumes, resulting from EES at different time points presented on Fig. 3A. In S1, inspired volumes progressively increased during the reconditioning period. Because inspiratory time was relatively constant, an increase in inspired volume was achieved by progressive increase in peak inspiratory flow rate. The maximum inspired volume achieved in this S1 was 186 ml. Maximum NIP was 1 cm H_2O. S1 was not able to maintain any breathing volumes in NIP 2 cm H_2O. Because inspiratory time was fixed, increase in inspired volume was achieved by increase in inspiratory flow rate. During the first few weeks after implantation, there was some increase in inspired volume production. As the duration of electrical stimulation during this period was relatively small, these changes were attributable in part to resolution of edema and inflammation at the electrode site.

4.3. *Breaths per minute evaluation on CPAP*

At the completion of the conditioning trial, inspired volumes were increased by approximately 100, 150 ml in S1 and 250, 450 ml in S2 respectively. S1 and S2 respectively tolerated 7–10 min and 40–45 min off mechanical support on CPAP. When pacing could no longer be tolerated, both subjects generally complained of feeling tired. S1 demonstrated some degree of dyspnea, although, maintained a PCO_2 of 35 mm Hg on mechanical ventilation. Arterial blood gases in the final minutes of pacing revealed a pH of 7.56 and a PCO_2 of 50 mm Hg, suggesting that acidosis was primarily responsible for the early development of respiratory discomfort. In S1, tidal volume gradually fell to approximately 75 ml, and PCO_2 gradually rose from 50 to 65 mm Hg, suggesting muscle fatigue. This subject showed improvements in breathing pattern (as initially had no breath). After the initial EES reprogramming to frequency 40 Hz, pulse width 210 μs, and amplitude 4.0 V, S1 was able to maintain 4–5 breaths per minute (bpm) on his own after 7 days. After 2 weeks, he maintained 10–12 bpm independently. S1 started showing self-breaths on CPAP system initially, unfortunately, later he developed gram negative (Klebsiella) pneumonia and his autonomic functions started diminishing, so we could not keep him on the CPAP system and went back to pressure control ventilator support. Overall, S1 was able to maintain 14–16 self-breaths on CPAP 20 weeks post stimulation in NIP 1 cm H_2O (Fig. 3B) and zero breathes in NIP 2 cm H_2O, indicating that S1 will require the mechanical ventilation support for a longer period.

On the contrary, S2 post stimulation (15th weeks onwards) tolerated a longer period off mechanical support in CPAP system more than 45 min with 20–22 bpm (Fig. 3B). The arterial blood gases parameters in S2 was maintained (PCO_2 at 33 mm Hg) post CPAP mode and sustained for 40–45 min. Later, PCO_2 level started to rise to 40 mm Hg, although, it was sustained after that for more than 1 h. S2 was able to maintain all the autonomic parameters and oxygen saturation. Due to the study design and study protocol, S2 was taken back to ventilator pressure support system. Overall, S2 maintained a good inspired volume in CPAP mode. During few attempts (5 attempts in 24 h, for about 10 min completely OFF ventilator), S2 was able to breathe himself and maintained saturations with consistent heart rate and blood pressure. S2 also demonstrated a good inspired volume more than 450 ml with breath frequency more than 20 in NIP 8.4 – 10.0 cm H_2O when he was on pressure support ventilation system.

4.4. *Diaphragm electromyography with and /or without stimulation (DEMG)*

Diaphragm EMG without EES did not show any positive outcome in both subjects (Fig. 3C, 3D and Fig. 4a, 4b, 4c, 4d). During EES, some EMG responses were observed in both patients. EMG electrodes were placed at the costal insertion of the diaphragm under the 8th, 9th, or 10th rib cartilage, distant from the major vessels, pleura, lungs, and abdominal viscera. A 50-mm monopolar needle electrode was used for this study. The ground electrode was placed on the sternum and a reference electrode was placed above the costal margin at the level of the needle insertion. The needle first passed through the skin and abdominal fascia, and at a depth of 1–2 cm, it reached the abdominal muscles. A rhythmic electrical activity of the muscles during inspiration was recorded as EMG bursts of 300–600 μV in amplitude, and 1–3 s in duration. EMG bursts were separated by regular intervals of electrical silence during passive expiration. In mechanically ventilated subjects, when the ventilator was temporarily disconnected for 60 s, a rhythmic electrical activity appeared with recorded inspiratory efforts, confirming the diaphragmatic location of the needle electrode. In a partially

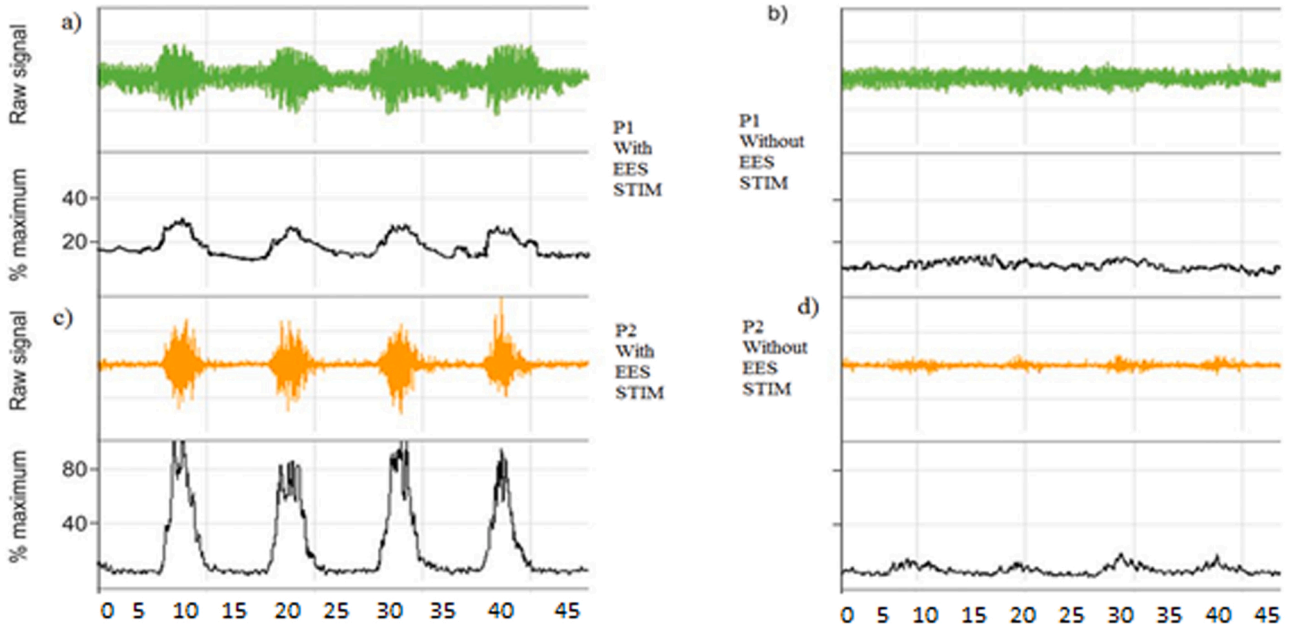

Fig. 4. Diaphragm EMG in S1 and S2 with or without EES stimulation. (4a) Demonstrates diaphragm EMG in S1 with EES and (4b) demonstrates for the same subject (S1) diaphragm EMG without EES. (4c) Demonstrates diaphragm EMG signals in S2 with EES stimulation and (4d) demonstrates diaphragm EMG in the same subject (S2) without EES. (Here Y axis refers the percentage of the EMG microvolt responses with and without stimulation and X axis refers the timeframe in seconds).

denervated diaphragm, fibrillation potentials or positive sharp waves were observed during the silent intervals. In subjects who could not tolerate weaning off the ventilator, we used a concentric needle instead of a monopolar for better localization. Stimulation of the phrenic nerve percutaneously at the neck, elicited a sharp diaphragmatic CMAP. This helped to determine what we presumed to be the proper location of the needle in the diaphragm (Fig. 4a & 4b for P1 and 4c & 4d for P2).

4.5. *Stimulation parameters for diaphragm electromyography and breaths per minute rate*

Diaphragm EMG without EES demonstrated no activity, while with EES clear EMG responses were observed. We attempted to calculate bpm, while subjects were on CPAP and we tested different stimulation settings over a period of 2 weeks (Table 3 for S1, and Table 4 for S2). In Table 1 and 2 we only present the settings with which we observed responses in EMG signals and bpm. Initially for 5 days stimulation pattern was 12 h ON and 12 h OFF state with cycling mode and stimulation amplitude was kept very low 1.0 V, pulse width was 100 μs and frequency was 10 Hz.

5. Discussion

The idea of using electrical stimulation to replace mechanical ventilation goes back to the 18th century (DiMarco et al., 1997b) when in 1786 Leopold Caldani reported that electrical stimulation of the phrenic nerve (PNS) could evoke diaphragm contraction and air movement in animals' lungs (DiMarco et al., 2004). This finding was replicated in a human post-mortem in 1818 by Andrew Ure (DiMarco et al., 2009b). In 1872, Guillaume-Benjamin and Duchenne reported that passing electrical current through moist sponges placed on the sternocleidomastoid muscles could evoke diaphragm contraction (DiMarco et al., 2009a). In 1948, Sarnoff et al., demonstrated the first practical applications of unilateral PNS, initially in animal models and then in a case series of bulbar poliomyelitis (DiMarco et al., 2004). Later, DiMarco, Kowalski, and colleagues revisited the concept of inspiratory intercostal pacing with EES and conceived a novel electrical stimulation pattern applied to the dorsal epidural surface of the spinal cord (Dimarco and Kowalski, 2013a). They demonstrated that high-frequency (100–300 Hz) EES (HF-EES) with a single epidural electrode at the second thoracic spinal segment (T2) can evoke a pattern in the inspiratory musculature in canine models of SCI (Dimarco and Kowalski, 2013a). Moreover, VTs were proportional to stimulus intensity, and maximum inspired volumes were reported at right below 1 L, which correlated with the maximal inspiratory capacity of the canine animal model (Dimarco and Kowalski, 2013a). Under normal conditions, activation of external intercostal muscles occurs along a rostrocaudal and dorsoventral gradient. Consequently, the contribution to the inspiratory drive is strongest in the rostral and dorsal portions and diminishes in caudal and ventral directions (DiMarco et al., 2005b). DiMarco et. al. in 2006 demonstrated the evidence that high frequency spinal cord stimulation at T2–5 segments can evoke recruitment pattern resembling spontaneous physiological breathing (DiMarco et al., 2005b).

Our results demonstrate that EES at the spinal segments related to intercostal muscles (T2–5) can help to maintain a substantial inspired volume. While inspired volume production by EES alone was not sufficient to comfortably sustain ventilation for a prolonged period, this approach may be useful in enhancing respiratory volumes in patients with suboptimal volume production via phrenic nerve pacing. Up to

Table 3
Stimulation settings for the diaphragm EMG responses with BPM for S1.

EMG responses & BPM	Frequency	Pulse width	Amplitude	Electrodes' Configuration
Latency ≥ 0.025 & BPM 2	20 Hz	210μs	3.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.045 & BPM 4	30 Hz	210 μs	4.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.075 & BPM 5	40 Hz	210 μs	5.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.15 & BPM 5	50 Hz	270 μs	5.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.25 & BPM 7	60 Hz	270 μs	5.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.30 & BPM 7	70 Hz	300 μs	5.0 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.35 & BPM 10	80 Hz	300 μs	5.0 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.43 & BPM 13	90 Hz	300 μs	5.0 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.50 & BPM 15	100 Hz	300 μs	4.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-

Table 4
Stimulation settings for the diaphragm EMG responses with BPM for S2.

EMG responses &	Frequency	Pulse width	Amplitude	Electrodes' Configuration
Latency ≥ 0.035 & BPM 3	20 Hz	210μs	3.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.055 & BPM 6	30 Hz	210 μs	4.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.095 & BPM 8	40 Hz	210 μs	5.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.25 & BPM 11	50 Hz	270 μs	5.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.35 & BPM 11	60 Hz	270 μs	5.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.40 & BPM 13	70 Hz	300 μs	5.0 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.45 & BPM 15	80 Hz	300 μs	5.0 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.50 & BPM 18	90 Hz	300 μs	5.0 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2:**8-, 9 + , 10-, 11 + , 12-
Latency ≥ 0.50 & BPM 22	100 Hz	300 μs	4.5 V	**Lead 1:**0 + , 1-, 3 + , 4-, 5 + **Lead 2-:**8-, 9 + , 10-, 11 + , 12-

now, about 12–15 articles have been published on effect of PNS to improve breathing patterns in respiratory compromised patients and most of them were published by DiMarco and his colleagues (Dimarco and Kowalski, 2013a; DiMarco et al., 2005b, 2006). One of these studies describes the outcomes of 5 patients with SCI treated with combination of PNS and EES of the same T2-T5 spinal segments (DiMarco et al., 2006). These subjects responded well to the combination of both therapies, although, none of them was successfully transferred to CPAP. In present study both patients were successfully transferred from mechanical ventilation to CPAP. Our results also suggest that with mechanical ventilation, volume and pressure control mode are not always optimal for the patients with prolonged ventilation, while EES at T2–5 was able to bring those patients to pressure support conditions considered to be beneficial for lungs. With EES both subjects demonstrated a decent breathing pattern and self-breathing. S1 was able to go up to 15 bpm on his own and S2 went up to 22 bpm and over the following weeks with adjustment of stimulation parameters (from 20 Hz to 100 Hz, from 2 V to 6 V stimulus amplitude, and from 210μs to 300μs with pulse width). The respiratory changes became visible, and patients were able to sustain their breath and maintained their inspired and expired breathing volumes. Following the previous findings, we also tested 300 Hz stimulation, however, with these settings no improvement was observed. In fact, with 300 Hz EES both patients' EMG responses started coming down and they became very fatigue with negligible diaphragm movements. Based on these findings, we hypothesize that high frequency EES at the T2–5 region affects the neuro-muscular junction (NMJ), which works via N-acetylcholine receptors and may trigger some relevant changes, so the patients' self-breathing pattern became negligible. This could be further confirmed with a biopsy of neuro-muscular junction and N-acetylcholine depletion as previously demonstrated it can cause breathing difficulties and lead to death (DiMarco et al., 2005b, 2002). In fact, in this study not only stimulus frequency was critical, but also the amplitude and pulse width had a major role on EES-enabled respiration. Overall, stimulation frequency of 20 Hz, pulse width of 210μs, and amplitude about 3.5 V caused consistent muscles response, although, patients were not able to sustain the tidal volume for a prolonged time.

In volume control or pressure control mode, the ventilation system should be monitored well and should not give more air or volume pressure to the lungs and mainly look for maintaining the tidal volume. Unnecessary air pressure via ventilation can cause barotrauma to the lungs and the efforts have to be directed to the ventilator volume control time, how much air pressure is given, and how much really patients need (Dimarco and Kowalski, 2013a). Accordingly, a combined approach with PNS consisted of implanted hook electrode via a laparoscopic surgical procedure, directly within each hemi diaphragm close to the phrenic nerve ending (DiMarco et al., 1999a) and side by side EES at T2 to T5 spinal segments to activate relevant intercostal muscles, could support patients dependent from mechanical ventilation (DiMarco et al., 2002). Combination of EES and diaphragm stimulation for ventilated patients demonstrated some benefits in the past (DiMarco et al., 1995, 1994, 2005a). Observed in this study asynchronous activation patterns may represent a correlation of 'quasi-physiological' motor recruitment patterns according to Henneman's size principle and could significantly enhance fatigue resistance of evocable respiratory performance. Although the underlying mechanism of this type of activation is not yet fully elucidated, Decima and von Euler in 1969 described the intercostal-to-phrenic reflex pathway within the spinal cord that may be involved in generating coordinated diaphragm activation (DiMarco et al., 2002). Complementary to the intercostal-to-phrenic reflex, additional afferent inputs from both inspiratory and expiratory intercostal muscles may help in mediating and coordinating excitatory signals to intra- and inter-segmental homonymous motor neurons via an intercostal-to-intercostal monosynaptic reflex. On the basis of this functional and anatomical understanding, DiMarco, Kowalski, and colleagues investigated the intrinsic spinal networks involved in inspiratory coordination, namely, the intercostal-to-intercostal and intercostal-to-phrenic reflex pathways (DiMarco et al., 1999a, 1997b). Nonetheless, it is possible that these reflex pathways may represent a major contribution in coordination of the normal intercostal muscles activity and chest wall motion with diaphragm contraction and represent the powerful targets for neuromodulation for clinical applications (DiMarco et al., 2006, 2002, 1999a).

6. Conclusion

Current evidence supports the use of EES at thoracic spinal segments T2-T5 to improve respiratory function in patients with high cervical SCI. Restoring the inspiratory capability beyond basic diaphragm contraction may provide a superior neuro-restorative pattern compared to the conventional stimulation techniques, although, further investigations of EES effect on respiratory function is warranted. Neuro-prosthetics devices may be beneficial not only for patients with high tetraplegia and complete respiratory failure, but also for those with partial respiratory and cough insufficiency who represent most of the population with SCI. Neuromodulation therapy for respiratory dysfunctions could be particularly helpful for patients on ventilation support. In neuro respiratory work-up, patients' selection is very important as acute cases can show better improvement than the chronic ones. This study included only 2 subjects and further multicenter collaborative studies along with proper training in the respiratory management teams are critical. In addition, accurate synchronization of the stimulation program with patient's respiratory volumes and breath frequency remains difficult and requires future solutions.

Ethics approval and consent to participate

This study was designed to analyze the spinal cord stimulation at the thoracic region (T2-T5) in respiratory compromised patients to restore their respiration and approved by all institutions' ethical committees.

Financial disclosures for previous 12 months

S.K.: Employment- Institute of Brain and Spine Hospital, India. **D.S.:** Employment- Institute of Brain and Spine Hospital, India. **A.K.T.:** Employment- Institute of Brain and Spine Hospital, India. **G.M.:** Employment- University of Cologne, Germany. **I.L.:** Employment- Mayo Clinic, USA. **P.M**: Employment- Institute of Brain and Spine Hospital, India.

Funding Sources and Conflicts of Interest

No funding has been taken for this study. The authors declare that there is no conflict of interest.

Author Roles

1. Research Project: A. Conception, B. Organization, C. Execution; 2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript Preparation: A. Writing the First Draft, B. Review and Critique.

S.K:.1A,1B,1C,2C, **D.S**:.1A,1B,1C,2C, **A.K.T**:.2B,3B, **G.M**:.2C,3B, **I.L**:.2C,3B, **P.M.:** 1A, 1C, 2A, 2B, 3A.

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