Research Article

Stimulation of abdominal and upper thoracic muscles with surface electrodes for respiration and cough: Acute studies in adult canines

James S. Walter^{1,4}, Joseph Posluszny^{1,2}, Raymond Dieter¹, Robert S. Dieter^{3,5}, Scott Sayers^{1,6}, Kiratipath Iamsakul¹, Christine Staunton¹, Donald Thomas^{1,2}, Mark Rabbat^{3,5}, Sanjay Singh¹

¹Research Services, Edward Hines Jr. VA Hospital, Hines, Illinois, USA, ²Surgery Services, Edward Hines Jr. VA Hospital, Hines, Illinois, USA, ³Cardiology Services, Edward Hines Jr. VA Hospital, Hines, Illinois, USA, ⁴Urology Departments, Loyola University, Stritch School of Medicine, Maywood, Illinois, USA, ⁵Cardiology Departments, Loyola University, Stritch School of Medicine, Maywood, Illinois, USA, ⁶Thoracic and Cardiovascular Surgery Departments, Loyola University, Stritch School of Medicine, Maywood, Illinois, USA

Objective: To optimize maximal respiratory responses with surface stimulation over abdominal and upper thorax muscles and using a 12-Channel Neuroprosthetic Platform.

Methods: Following instrumentation, six anesthetized adult canines were hyperventilated sufficiently to produce respiratory apnea. Six abdominal tests optimized electrode arrangements and stimulation parameters using bipolar sets of 4.5 cm square electrodes. Tests in the upper thorax optimized electrode locations, and forelimb movement was limited to slight-to-moderate. During combined muscle stimulation tests, the upper thoracic was followed immediately by abdominal stimulation. Finally, a model of glottal closure for cough was conducted with the goal of increased peak expiratory flow.

Results: Optimized stimulation of abdominal muscles included three sets of bilateral surface electrodes located 4.5 cm dorsal to the lateral line and from the 8th intercostal space to caudal to the 13th rib, 80 or 100 mA current, and 50 Hz stimulation frequency. The maximal expired volume was 343 ± 23 ml ($n=3$). Optimized upper thorax stimulation included a single bilateral set of electrodes located over the 2nd interspace, 60 to 80 mA, and 50 Hz. The maximal inspired volume was 304 ± 54 ml ($n=4$). Sequential stimulation of the two muscles increased the volume to 600 ± 152 ml ($n=2$), and the glottal closure maneuver increased the flow.

Conclusions: Studies in an adult canine model identified optimal surface stimulation methods for upper thorax and abdominal muscles to induce sufficient volumes for ventilation and cough. Further study with this neuroprosthetic platform is warranted.

Keywords: Spinal cord injury, Respiration, Functional electrical stimulation, Respiratory distress, Cough, Neuroprosthetic

Introduction

Following severe cervical spinal cord injury (SCI), mechanical ventilation is usually required. Mechanical ventilation methods with and without endotracheal tubes have been improving and include protocols such as bi-level positive airway pressure ventilation, and mechanical insufflation-exsufflation.¹⁻⁴ These methods, however, continue to have risks for respiratory insufficiency, atelectasis, bronchospasm, pulmonary edema, and pneumonia.^{2,4} Functional electrical stimulation (FES) of diaphragm muscles for inspiration and ventilation is an important alternative to mechanical

ventilation. A widely used device for diaphragm stimulation is the NeuRx® Diaphragm Stimulator (Synapse Biomedical Inc, Oberlin, OH, USA), which was developed at Case Western University and Cleveland FES Center (USA) and uses intramuscular Permaloc® electrodes implanted in the diaphragm muscle. Although the majority of individuals with SCI who are implanted with this device obtain 24-hour ventilation, there are important limitations due to paralysis of upper thorax and abdominal muscles.⁵⁻⁷

Paralysis of the upper thorax results in inward movement of the chest during diaphragm stimulation, which reduces the inspiratory volume.⁵⁻⁷ Methods to expand the upper chest with FES are under intense investigation

Correspondence to: James S. Walter, Edward Hines Jr. VA Hospital (151), 5000 South 5th Avenue, Hines, IL 60141, USA. Email: James.Walter@va.gov

and include electrodes applied to the upper thoracic spinal cord, electrodes in the upper thoracic intercostal muscles, and external magnetic coils for stimulation of the upper thoracic spinal cord.^{8–14,27,28} None of these methods, however, have been commercialized. Stimulation with surface electrodes placed over the upper thorax are promising because they can be easily and safely applied.¹² In our initial experience with this method, results were unsatisfactory because of induction of excessive forelimb movement and use of a juvenile canine model.¹¹ In the current study, further testing was conducted using an adult canine model and high stimulating current on a single set of bilateral surface electrodes over the upper thorax.

Although abdominal contractions are not normally recruited for tidal volume, they are needed during exercise and respiratory distress.¹ Abdominal and lower thorax muscle contractions contribute to the inspired volume after stimulation when, during muscle relaxation, the elastic recoil of the lungs and/or the abdominal and thorax walls returns the lung volume to the functional reserve capacity.¹ Abdominal contractions are also required for coughing. Following SCI paralysis there is a particular need to induce abdominal contractions for ventilation and cough, and this could be applied in conjunction with diaphragm stimulation. Several abdominal FES methods have demonstrated high abdominal pressures that result in large expiratory volumes. These methods include peripherally located surface and implanted electrodes, centrally located electrodes adjacent to the lower thoracic spinal cord, and magnetic stimulation over the lower thoracic spinal cord.^{12,15–24} Comparing these methods, FES with surface electrodes placed over abdominal muscles is promising. In our initial studies using this method, results were limited by less than optimal stimulation and the use of a juvenile canine model. In the current studies, we conducted further tests evaluating stimulation parameters, electrode number and locations, and arrangements of bipolar sets of electrodes; the adult canine model was also used. Finally, tests with combined extradiaphragmatic muscles were conducted along with a model of glottal closure for cough. All of the stimulation tests used with a computer-controlled, 12-Channel, Neuroprosthetic Platform (Synapse Biomedical, Inc).

Methods

Anesthesia, respiratory instrumentation, apnea, and stimulation techniques

Six adult male, mixed-breed canines at least eight months old and weighing 27.9 ± 0.7 Kg (mean $\pm$ SEM) were obtained from a commercial vendor (Oak Hill

Genetics; Ewing, IL, USA).^{11,27} Anesthesia was initiated with intravenous catheter propofol (6 mg/kg) using a cephalic vein catheter. Sevoflurane (1.5–3%) was then delivered through an endotracheal tube connected to a ventilator (Dräger Anesthesia Rebreathing Ventilator; Louisville, KY, USA). Anesthesia also included fentanyl citrate administered intravenously at the rate of five to ten micrograms per kilogram per hour. The amount of anesthetic was adjusted in each animal to maintain a deep level of surgical anesthesia. Atropine was used to reduce respiratory secretions during surgery as previously described.¹¹

Anesthetized animals were instrumented for respiratory monitoring. Endotracheal tube pressure was recorded at the distal end outside of the canine, and esophageal pressure was measured at a location just rostral to the diaphragm utilizing a small balloon-tipped tube filled with water.¹¹ A similar tube was advanced into the colon to a location 23 cm from the anus that was used to monitor abdominal pressure. A lead II electrocardiogram (EKG) was recorded using wire hook electrodes placed under the skin in the right upper and left lower limbs. The EKG recording was analyzed by the computer program to display heart rate (ADInstruments). Effects of stimulation on heart rate was only determined during the initial abdominal recruitment test because electrical field interference during the stimulation prevented this measurement during other tests.¹¹ Effects of upper thorax stimulation on ventricular arrhythmia was assessed during post hoc analysis of the EKG recordings. Only ventricular contractions could be seen in the EKG recording during and shortly after stimulation because the electrical interference prevented assessment of atrial contractions. Ventricular arrhythmia was assessed by the occurrence of premature ventricular contractions or a series of high frequency ventricular contractions. The assessment could not determine the origin of the arrhythmia, atrial or ventricular, because of interference in the atrial EKG record. All recordings were obtained digitally (16-channel recorder, ADInstruments, Inc) and displayed on a computer screen.

The ventilator used a closed airway system that included a waste gas and anesthetic scavenger along with a calcium carbonate scrubber that removed the CO₂ gas prior to rebreathing.^{11,27} The airway tubing included inspiratory and expiratory tubes connected through a 'Y' connector. Between the animal's endotracheal tube and the 'Y' connector there was an air filter for absorbing moisture, a pneumotachometer (Model 300L; ADInstruments, Inc; Colorado Springs, CO, USA) for recording flow and a short piece of rubber tubing that was used for clamping in a model of glottal closure.

Respiratory flow was integrated to record the inspiratory and expiratory volumes (ADInstruments). The ventilator included valves to insure that gases only flowed in one direction through the respiratory tubing using two modes of operation: 'Ventilation' and 'Bag.' The 'Ventilation' mode was used before and after stimulation test periods and included delivery of 100% O₂ at 2 L/min. The 'Bag' mode was used during stimulation tests. This mode separated the ventilator bellows from the airway and included a 1-liter bag. The bag was adjusted to be half-full prior to the start of stimulation tests to prevent the bag from becoming fully expanded or contracted; conditions that could affect respiratory responses to stimulation. To avoid insufflation due to a high rate of O₂ gas flow from the gas tank, the flow was reduced to 200 ml/min. These methods resulted in no spontaneous inspiratory attempts during testing periods without stimulation.²⁷

During surgery the animals were ventilated at 12 ventilations per minute (rpm) with the tidal volume adjusted to obtain a normal end tidal volume pCO₂ of 40 mmHg. Before electrical stimulation-tests, the animals were hyperventilated at 25 rpm for two minutes by adjusting the respiratory rate dial on the ventilator to drive the end-tidal CO₂ down to less than 35 mmHg. Following switching the ventilator to the 'Bag' mode, the respiratory apnea lasted longer than two minutes during which time, stimulation-tests were conducted. Stimulation records within 10 seconds of a spontaneous inspiration were excluded from analysis.^{11,27}

Surface electrodes were 4.5 cm square and were composed of conductive black rubber (4.5×4.5 cm, Medtronic, Inc; Minneapolis, MN, USA).¹⁰ Frequent wetting of the skin-electrode interface with isotonic saline assured good electrical conduction (Fig. 1). To further maintain a wet interface, a single layer of gauze was placed at the interface. Electrical stimulation was delivered with a 12-Channel Neuroprosthetic Platform (Synapse Biomedical) that was controlled by a computer.¹¹ Stimulation pulses were balanced biphasic and constant-current, and each phase of the biphasic pulse was a square wave of 100 μs duration with a 100 μs delay between alternative pulses. Selectable from the computer screen were stimulating currents (1 to 100 mA; the total stimulating current is the sum of the currents from each of the applied stimulation channels), ventilations per minute (rpm, 2 to 20), stimulation frequency (10 to 50 Hz), and stimulation period (0.4 to 1.5 seconds).

Stimulation optimization protocols to induce maximal respiratory volumes included comparisons of different stimulation parameters, and electrode numbers, locations, and arrangements. During each testing

Figure 1 Photograph of left lateral view of the canine chest and abdomen with surface electrodes placed on the skin at intercostal spaces marked in black ink. For abdominal stimulation, four electrodes are shown over the lower thoracic and abdomen with the center of the electrodes located 4.5 cm dorsal to the lateral line and at the 7th intercostal interspace to 4.5 cm caudal from the 13th rib. For upper thoracic stimulation, one electrode is shown over the 2nd intercostal space just ventral to the axilla. White gauze over the 4.5 square electrodes to maintain moisture was deleted from the image. Bilateral electrodes were used in all of the tests.

period, two to four stimulations were conducted and then a parameter was changed with and an additional two to four stimulations performed. During current-response tests, several different currents were usually conducted during a single apneic period. Stimulation conditions that induced the largest expired volume in the comparison tests were used in subsequent tests. Differences in responses were analyzed by a Student's *t*-test for paired data. A probability of $P < 0.05$ was considered as statistically significant. Three different currents were used in the recruitment test for abdominal muscles and results from these tests were compared by Analysis of Variance followed by post hoc analysis using the paired Student's *t*-test and the Bonferroni correction factor of $P = 0.017$ ($0.05/3$). Results for fewer than six animals were obtained for some tests due to stimulation or recording difficulties or due to the late introduction of tests. Pilot tests with only two results were not analyzed statistically.

Abdominal muscle stimulation

Optimization tests for maximal expirations were conducted with electrodes placed over lower thoracic and abdominal muscles. Current-response tests were conducted first and included methods that we have previously shown to be effective.^{11,27} Three bilateral sets of electrodes were used that had one pole of the bipolar set of electrodes on one side of the animal and the other pole on the other side. Electrodes were equally spaced along the lateral line and were connected to separate stimulation channels. The lateral line divides the ventral from the dorsal halves of the lower thoracic

and abdominal walls and is the same as the midaxillary line.¹¹ The electrodes were placed at the 8th and 10th intercostal spaces as well as 4.25 cm caudal to the 13th rib (Fig. 1 shows 4 electrodes that are 4.5 cm dorsal to the lateral line). Stimulation parameters were 50, 80 and 100 mA, 1.4-second stimulation period, and 50 Hz frequency. Responses to stimulation were measured as the peak expired volume and peak abdominal pressure. These values usually occurred at the end of the stimulation period.

The second abdominal test evaluated the effects of moving the most rostral electrode from the 8th to the 7th intercostal space. The third test compared stimulation locations on the lateral line to 4.5 cm dorsal to the lateral line. The fourth test evaluated the effects of increasing electrode surface area; the original set of electrodes was compared to electrodes with twice the surface area using a second set of electrodes of the same area and connected to the original set. The fifth test assessed the effects of adding a fourth set of bilateral electrodes. The sixth test compared the bilateral electrode arrangement, which had been used to this point to a unilateral arrangement in which both electrodes in a bipolar set were on the same side of the animal. Four channels of stimulation were needed to conduct this test because unilateral stimulation was best conducted with two bipolar sets of electrodes on each side of the animal, and as this protocol was added late in the study, testing was only conducted in three animals. In all of these tests, a six-point rating scale of back-arching was used: none, slight (threshold), slight-to-moderate, moderate, moderate-to-strong and strong.

Upper thorax muscle stimulation

Optimization tests to obtain a maximal inspiration with not more than slight-to-moderate forelimb movement were conducted with surface electrodes. This protocol included methods that we had previously used such as current-response as well as new methods such as higher stimulating currents on a single bilateral set of surface electrodes.¹¹ Stimulation test locations were located just ventral to the axilla and from the 1st through the 4th intercostal spaces (Fig. 1). Stimulation parameters included 50 Hz, a 1.6-second stimulation period, and currents from 30 to 100 mA with 10 mA increments.¹¹ The rating of forelimb movement used the immediate above scale.

Combined extradiaphragmatic muscle stimulation

In the first test, upper thoracic stimulation was followed immediately by lower thoracic and abdominal muscle stimulation.^{11,27} The induced volume, a 'stimulated

expiratory volume,' was measured from maximal inspiration at the end of upper-thoracic stimulation to the maximal expiration at the end of abdominal stimulation. This volume is analogous to a respiratory tidal volume. Effective stimulation parameters determined above were used. For the second test, a model of glottal closure was used and effects on maximal expiratory flow was measured. A flexible tube in the airway line was manually clamped at the transition between upper thoracic and abdominal stimulation. The time for clamping was based on laboratory tests where it took the investigator approximately 200 ms to clamp the tubing when the computer-controlled light signal came ON and 200 ms to release the clamp when the light went OFF. During the current animal test, the light signal was turned ON 200 ms before the onset of abdominal stimulation and OFF at the start of abdominal stimulation.

Results

Abdominal muscle stimulation

A recruitment test using three bilateral sets of surface electrodes located along the lateral line is shown in Figure 2. Increasing the stimulating current from 50 to 80 mA induced a higher peak abdominal pressure as well as a greater expired volume. Further increasing the current to 100 mA induced no greater expired volume; thus, 80 mA was the most effective current for this test. Also shown in Figure 2, the abdominal pressure tracing is irregular at the onset of stimulation indicating unphysiological rapid abdominal wall contraction. The heart rate tracing during stimulation demonstrates an increase of approximately 2 bpm that was not sustained after stimulation.

Results for the six optimization tests for maximal abdominal pressure and expired volume are presented in Table 1A to 1F. The current recruitment test demonstrated that current greater than 50 mA significant increase abdominal pressures, but had no effect on volume (50, 80 and 100 mA; Table 1A). During this test the heart rate increased 2.3 ± 0.3 bpm ($n = 4$, not shown in Table 1), which returned to pre-stimulation levels shortly after the end of the test. Based on the largest induced expiratory volume obtained in individual animals, a single current of 80 mA continued to be used in three animals and 100 mA in three others. In the second test, the location of the electrode at the 8th intercostal space was moved to the 7th space, which did not result in a significant change in volume (Table 1B). Based on the results in individual animals, the 8th interspace location continued to be used in three animals and the 7th interspaces in three others.

Figure 2 Recruitment test with surface electrodes placed over lower thorax and abdominal muscles. Increasing current induced greater expiratory responses. Eighty mA was optimal because 100 mA did not induce a greater expired volume. Stimulation included three bilateral sets of electrodes located along the lateral line and caudal to the 13th rib, and over the 10th and 8th intercostal spaces, 50 Hz, 1.4-second period, and at the currents indicated below the traces.

For the third optimization test, the location of the three electrodes on the lateral line was compared to a location 4.5 cm dorsal to the lateral line. The dorsal location resulted in a significant, $144 \pm 11\%$, increase in expired volume (Table 1C). This increased volume was associated with a significantly reduced abdominal pressure that was only $84 \pm 5\%$ as high as the pressure obtained from the lateral line. The dorsal location also induced slight-to-moderate upward arching of the middle of the back that was not present with stimulation along the lateral line. For all six animals the dorsal electrode placements were used in the last three tests. The fourth test compared the three bilateral sets of electrodes to those with twice the surface area, and there was no significant change in expired volume; thus, the standard electrode surface area (a single square of 4.5 cm) was used subsequently (Table 1D). The fifth test compared three vs four sets of bilateral electrodes and there was no significant change in volume (Table 1E); thus, three sets of electrodes was a minimal number that induces a maximal expired volume. The sixth and last test compared a unilateral electrode arrangement to the bilateral electrode arrangement used thus far. Bilateral stimulation induced significant increases in pressure and volume by $316 \pm 103\%$ and $441 \pm 141\%$

Table 1 Six optimization tests were conducted with abdominal surface stimulation for abdominal pressure and maximal expiratory volume. Stimulation tests include effects of current and electrode locations, number, size, and configurations. Tests conducted with 50 Hz, 1.4 s stimulation period, and the 4.5 cm square surface electrodes. $\pm$ represents standard error of the mean (SEM).

	Abdominal Pr (cm H ₂ O)	Expired Volume (ml)
A. Recruitment Test on Lateral Line^a		
50 mA	37 ± 7	210 ± 15
80 mA	$67 \pm 8^*$	258 ± 28
100 mA	$77 \pm 7^{**}$	262 ± 38
B. Rostral Sets of Electrodes compared at 7th and 8th Interspaces^b		
T7	57 ± 7	253 ± 21
T8	61 ± 9	261 ± 21
C. Electrodes on Lateral Line Compared to 4.5 cm Dorsal^c		
Lateral Line	60 ± 9	252 ± 3
4.5 cm dorsal to lateral line	$50 \pm 7^*$	$358 \pm 31^*$
D. Initial Compared to Twice the Electrode Surface Area^d		
Single Area	55 ± 8	333 ± 41
Double Area	55 ± 8	322 ± 41
E. Three Compared to Four Sets of Electrodes^d		
Three Electrodes	59 ± 9	363 ± 28
Four Electrodes	65 ± 8	338 ± 34
F. Bilateral Electrode Configuration Compared to Unilateral^e		
Bilateral	58 ± 3	343 ± 23
Unilateral	$19 \pm 7^*$	$133 \pm 30^*$

^aThree bilateral sets of electrodes located at the 8th, 10th interspaces and 4.5 cm caudal to 13th rib (*50 mA to 80 mA, $P = 0.0004$; **50 to 100 mA, $P = 0.0014$; $n = 6$).

^bStimulating currents were used that were optimal in individual animals from Section A ($n = 6$).

^cStimulation methods were used that were optimal in individual animals from Section B, and the dorsal location was 4.5 cm from the lateral line (*abdominal pressure, $P = 0.008$, *expired volumes, $P = 0.008$; $n = 6$).

^dStimulation methods were used that were optimal in individual animals from Section C (for Section D, $n = 5$; for Section E, $n = 6$).

^eStimulation methods were used that were optimal in individual animals from Section E; however four bipolar sets of electrodes were used (see text). (*abdominal pressure, $P = 0.034$; *expired volumes $P = 0.01$; $n = 3$).

respectively ($n = 3$) demonstrating that the bilateral method is superior (Table 1F). The optimized expiratory volume at the end of the six tests was 343 ± 23 ml (Table 1F, $n = 3$).

Upper thorax muscle stimulation

A recruitment test with a single set of bilateral surface electrodes located at the 2nd intercostal space is shown in Figure 3. The 80 mA current induced the largest inspiratory volume of 500 ml; however, this current induced an unacceptable, moderately strong, forelimb movement. The 60 mA current induced an inspiratory volume of 280 ml with an acceptable slight-to-moderate forelimb movement; therefore, 60 mA was determined to be the optimal current. Also, these inspiratory volumes were caused by current-dependent, graded decreases in negative esophageal and tracheal pressures reaching –

Figure 3 Recruitment test with surface electrodes placed over upper thoracic muscles. Increasing stimulating current induced greater inspiratory responses. Sixty mA was optimal because of the large inspired volume without more than slight-to-moderate forelimb movement. Stimulation included a single bilateral set of electrodes located just ventral to the axilla at the 2nd intercostal space, 50 Hz, 1.6-second stimulation period, and at the currents indicated below the traces.

5 cm H₂O. The pressure tracings also show irregular responses at the start of stimulation indicating physiological and rapid movements of the upper chest wall.

Recruitment responses with electrodes located at the 1st through 4th intercostal spaces are summarized in Table 2A–2D. At the 1st intercostal space, a 300 to 400 ml inspiratory response was obtained that was associated with slight-to-moderate forelimb movement; however, there was a risk for pectoral and shoulder muscle contractions as evidenced by forelimb-movement (Table 2A). Complete test results for four animals at the 2nd intercostal space are shown in Table 2B. At the lower currents of 40 to 60 mA there was no forelimb movement and the inspired volumes ranged from 60 to 325 ml. At 60 to 80 mA there was slight-to-moderate forelimb movement and the inspired volume increased to 115 to 400 ml; the mean inspiration was 304 ± 54 ml (not shown in Table 2). The peak negative endotracheal tube pressures associated with these volumes was -6 ± 1 cm H₂O (mean not shown in Table 2B; $n = 3$). Higher stimulating currents induced excessive forelimb movement and, in some cases, greater inspirations. Testing at the 3rd intercostal space produced an adequate inspiratory volume with only slight-to-moderate forelimb movement. Stimulation at the 4th intercostal space induced less inspiratory volume with reduce or no risk of unwanted forelimb

movement (Table 2C, 2D). Review of the EKG recordings demonstrated no evidence of cardiac arrhythmia.

Combined extradiaphragmatic muscle stimulation

A recording of sequential upper thoracic followed by abdominal stimulation is shown for the first two stimulations in Figure 4. Upper thoracic stimulation induced a large inspiratory volume followed immediately by the expiratory response to abdominal stimulation. The measured ‘stimulated expiratory volume’ is shown by the arrow in the recording. The endotracheal tube pressures reached 14 ± 6 cm H₂O at the time of the ‘stimulated expiratory volume’ demonstrating high airway resistance. The final two sequential stimulations shown in Figure 4 included a short period of airway tube clamping as a model of glottal closure. This maneuver caused a large increase in esophageal and endotracheal tube pressures but only a small increase in the maximal expiratory flow. The maneuver also caused a slight decrease in the induced ‘stimulated expiratory volume’ whereas abdominal pressure was unchanged.

Complete results from this protocol were limited to two animals (Table 3). A large ‘stimulated expiratory volume’ of 600 ± 152 ml and flow -1.3 ± 0.3 L/s were associated with a tracheal pressure of 13.5 ± 5.6 cm H₂O pressure (values not shown in Table 3). The tracheal tube maneuver caused a small decrease in

Table 2 Optimization of surface stimulation was conducted over the upper thoracic to obtain negative endotracheal tube pressure and maximal inspiratory volumes. Tests included current recruitment and electrode placements at different intercostal spaces that were just ventral to the axilla. Tests conducted with 50 Hz, 1.6-second stimulation period, and the 4.5 cm square surface electrodes^a

Current (mA)	Forelimb movement	Tracheal Pr (cm H ₂ O)	Inspired Volume (ml)
A. 1 st Intercostal Space			
Animal 1			
70	<i>Slight-to-moderate</i>	-6	280
Animal 2			
80	<i>Slight-to-moderate</i>	-10	380
B. 2 nd Intercostal Space ^b			
Animal 1			
60	None	-5	300
80	<i>Slight-to-moderate</i>	-10	400
100	Moderate-to-strong	Stopped stimulation	
Animal 2			
50	None	NR	200
60	<i>Slight-to-moderate</i>	NR	300
70	Moderate	NR	280
Animal 3			
40	None	-1	60
50	Slight	-2	115
60	<i>Slight-to-moderate</i>	-3	115
Animal 4			
60	None	-6	325
70	<i>Slight-to-moderate</i>	-6	400
80	Moderate	-8	500
C. 3 rd Intercostal Space			
80±6	<i>Slight-to-moderate</i>	-4.5 ± 2.5	233 ± 69 (n = 3)
D. 4 th Intercostal Space			
70±6	None	-3 ± 1	138 ± 26 (n = 3)

^aSummary results in bold; individual animals in normal weight font.
^b*Italic* used for results for the greatest allowed forelimb movement of slight-to-moderate. See text for mean result.

the ‘stimulated expiratory volume’ and the tracheal pressure and flow were 190 ± 15% and 127 ± 14% respectively compared to without the maneuver (statistical comparisons not conducted).

Discussion

Abdominal muscle stimulation

Abdominal stimulation in paralyzed patients has been conducted with three different methods: abdominal surface electrodes,^{19–23} lower thoracic spinal cord electrodes,^{12,15–18} and magnetic stimulation over the lower thoracic spinal cord.¹⁴ Among these methods, the use of surface electrodes is promising because they are

Figure 4 Respiratory responses shown for sequential stimulation of upper thoracic followed by abdominal muscles. Tests were conducted with and without a short period of clamping of the tracheal tube as a model glottal closure. The flow tracing shows slightly greater peak expiratory flow for the closure maneuver (a versus b). The respiratory volume tracing shows the ‘stimulated expired volume’ (marked with arrow). This volume decreased slightly during the closure maneuver. Abdominal pressures shown in the third tracing was little changed by the maneuver. Esophageal and tracheal pressures recorded were greatly increased by the maneuver, which caused the increased expiratory flow. Stimulation was conducted with optimal methods determined during prior tests and included 50 Hz, and 1.4-second stimulation periods noted by the black bars at the bottom of the figure. Abdominal stimulation used three bilateral sets of electrodes located dorsal to the lateral line and 100 mA. Upper thoracic stimulation used a single set of electrodes at 2nd interspace and 70 mA.

noninvasive and easily applied. Surface stimulation was currently optimized in adult (8 month old) canines that were anesthetized and respiratory apneic. Optimized results included three sets of bilateral electrodes located 4.5 cm dorsal to the lateral line and from the 7th or 8th intercostal space to caudal to the 13th rib, 80 or 100 mA current, and 50 Hz stimulation frequency. The maximal expired volume was 343 ± 23 ml (n=3, Table 1F). This volume is larger than we previously reported using similar stimulation methods in juvenile five-month-old canines; thus, adult canines with their greater muscle mass are a better animal model.¹¹ In addition, the current maximal expiratory volume is somewhat greater than the maximal value of 257 ± 31 ml reported for these animals using three or four bilateral sets of intramuscular electrodes implanted in lower thorax and abdominal muscles.²⁷ Finally, our maximal expiratory volumes are consistent with reports by lower thoracic stimulation of the spinal

Table 3 Combined extradiaphragmatic muscle stimulation results with and without a short closure of the tracheal tube as a model of glottal closure. The 'expired stimulated volume' is presented as the stimulated volume. The maneuver increased the peak flow and tracheal tube pressure. Stimulation methods were used that had been shown to be effective in individual muscles.

	No Tracheal Tube Closure			Short Tracheal Tube Closure		
	Stimulated Vol (ml)	Tracheal Pr (cm H ₂ O)	Flow (L/s)	Stimulated Vol (ml)	Tracheal Pr (cm H ₂ O)	Flow (L/s)
Animal 1 ^a	750	19	1.6	710	39	1.8
Animal 2 ^b	450	8	1.0	400	14	1.4

^aResults for animal shown in Figure 4; abdominal stimulation in both animals included three bilateral sets of electrodes located dorsal to lateral line, 100 mA; Upper thoracic stimulation: single set of electrodes at 2nd thoracic interspace electrode, 80 mA.

^bAbdominal stimulation included three bilateral sets of electrodes dorsal to the lateral line and including 8th interspace, 100 mA; Upper thoracic stimulation: single set of electrodes at second thoracic interspace, 70 mA. The stimulated volume is the 'expired stimulated volume'.

cord or adjacent to vertebral foramina in canine models.^{12,24} For clinical comparison, the optimized current of 80 or 100 mA applied to three sets of the surface electrodes, a total current of 240 or 300 mA, is similar to the current used for surface electrodes in patients with SCI.^{19–23}

Among the six abdominal optimization tests, two results were outstanding. The first compared electrodes located on the lateral line to 4.5 cm dorsal to the lateral line. The dorsal location induced the greater expiration even though the abdominal pressure was lower (Table 1C). Two competing factors may explain this result. First, the reduced abdominal pressure from the dorsal location may have been caused by less contraction of lower thoracic oblique muscles. Such an effect would be expected to reduce the expired volume. Second, the slight-to-moderate arching only from the dorsal location probably increased the movement of abdominal contents upward against the diaphragm increasing the expired volume. Because the dorsal location induced the greater volume, the back arching appears to be a strong effect. It should be discussed that these dorsal locations are different from clinical reports where locations along a diagonal line extending from a dorsal lateral area caudal to the 13th rib upwards toward the 8th interspace near the sternum are most effective.^{19,22,23} Thus, a comparison tests between these two results for effective electrode locations is warranted.

The second important finding was with the bipolar sets of electrodes that bilateral arrangements were far superior to unilateral arrangements (Table 1F). Two factors may contribute to this finding. First, the spread of the electrical field, in this case ions in the body, to stimulate muscle nerves is enhanced by a wide separation of electrodes, and the bilateral arrangement provides greater separation than the unilateral arrangement. Second, adjacent electrodes of the same polarity will force the electrical fields deeper into the tissue to

stimulate the nerves, and the bilateral arrangement uses adjacent electrodes of the same polarity whereas the unilateral arrangement does not.

Another finding was that heart rate was slightly increased during abdominal stimulation, which is not an unexpected physiological response for two reasons: first, the increased abdominal pressure during abdominal stimulation will cause an overall decrease in venous return to the right side of the heart and therefore decreased blood pressure; second, the lower blood pressure will cause a baroreceptor reflex response that will increase heart rate. Another finding was that the area of the surface electrodes had little effect on the expiratory response. The above results extend our recommendations on optimal methods for abdominal surface stimulation.¹¹ In the prior studies, we demonstrated that simultaneous channel stimulation is far superior to staggered channel stimulation, 50 Hz stimulation frequency is more effective than 20 Hz, and that current ramping greatly reduces the muscle jerking effects of stimulation.¹¹ All of these methods should be taken into consideration when planning abdominal muscles stimulation.

Upper thoracic muscle stimulation

Studies in patients with SCI for upper thorax stimulation and inspiratory effects includes electrodes implanted on the spinal cord and external magnetic stimulation over the back.^{9,10,12,14,16} This promising work continues in technology transfer programs. To our knowledge, this is the first report of successful stimulation of upper thoracic external intercostal muscles using surface electrodes in an animal model. Optimized upper thorax stimulation included a single bilateral set of electrodes located over the 2nd interspace, 60 to 80 mA, 50 Hz, and no more than slight-to-moderate forelimb movement. The maximal inspired volume was 304 ± 54 ml (Table 1F), and when lower

stimulating currents were used the forelimb movement could be avoided but the inspiratory volume was also reduced. These responses are superior to our prior results for this goal, which is probably due to the current use of higher stimulating currents on a single set of bilateral surface electrodes and the use of larger adult canines.¹¹ In addition, this maximal inspiratory volume is somewhat lower than the maximal inspiratory volume of 409 ± 91 ml obtained from these animals using three or four sets of bilateral intramuscular electrodes in the upper thorax.²⁷ Finally, this inspiratory volume is consistent with results by others using spinal cord stimulation in canine models.^{8,12,13,17}

Testing with surface electrodes over the upper thorax in individuals with SCI with paralysis of the upper thorax is warranted because of the positive results obtained here and because of the minimal risks associated with these electrodes. To avoid painful stimulation, testing will be limited to cervical SCI with no skin sensation in this area. Another concern is the risk of heart arrhythmia. No heart arrhythmia was induced during the current tests. Also promising for demonstrating limited risk in this area, in the second report from these animals that used 12 bilateral sets of intramuscular electrodes implanted in the upper thorax and high stimulating current, a high safety factor from heart arrhythmia was reported.²⁷ Finally, the use of high stimulating current in the upper thorax may be needed for certain conditions such as maximal cough or during respiratory distress. Such currents would be expected to elicit involuntary arm movement, which could be managed with the use of soft arm restraints.

Combined extradiaphragmatic muscle stimulation

Although combined muscle stimulation tests were only accomplished in two animals, the 'stimulated expired volume' and peak flow were high (Table 3). The values are consistent with combined extradiaphragmatic muscle stimulation results obtained by other investigators using implanted spinal cord electrodes.^{8,12,16} The glottal closure maneuver for cough increased the tracheal expiratory pressures and peak flow. The light signal for this procedure could be used by patients with SCI to improve their cough response.

Study limitations

The current model of SCI respiratory paralysis included spinal intact canines under general anesthesia and respiratory apnea following hyperventilation. Although this model is widely used in respiratory FES testing, there are limitations. The current model will have reduced spinal reflex activity and no muscle wasting as

occurs after SCI.^{12,27} Another limitation in these studies was high airway resistance which probably reduced the respiratory volumes elicited by stimulation. Endotracheal tube pressures were measured at the distal end, and if this tube were open to air, the pressure would be zero. However, pressures up to 19 cm H₂O (Fig. 4) or higher occurred and represent resistance from respiratory tubing, air filter, pneumotachometer and ventilator. During the 9-hour surgery, accumulation of airway secretions probably occurred to increase this resistance even though atropine was administered to reduce secretions.²⁷

Not conducted during these studies was extended pacing tests to produce minute ventilation results, for example, 5 to 30 min of stimulated-pacing at 16 rpm. These studies are needed to determine if muscle fatigue occurs, which would decrease the induced tidal volume. In a prior report, however, we demonstrated that these volumes were maintained during extradiaphragmatic muscle pacing for 3 min.¹¹ Another reason for extended ventilatory pacing studies is assessment of metabolic demand and benefits from surface stimulation. A significant metabolic benefit was shown by others during long term respiratory pacing of extradiaphragmatic muscles using spinal cord electrodes.¹²

Future directions

There is a continuing need to develop improved methods to assist with ventilation and cough following SCI, particularly in individuals with tetraplegia.²⁷ Current results with surface electrodes and the 12-Channel Neuroprosthetic Platform extends our prior findings toward this goal.^{11,25-31} Further study is needed, because some of the current studies were limited to only two or three animals and other limitations cited above. Clinical testing of some of the current methods, however, is warranted because surface electrode stimulation is widely used in patients with SCI.¹⁹⁻²³ Such testing depend on the level of spinal cord injury because the stimulation has to be applied in non-sensate areas. For SCI at low spinal cord levels, surface stimulation is limited to lower thoracic and abdominal muscles. For individuals with cervical level SCI, surface stimulation could be applied to both extradiaphragmatic muscles for expiration. For individuals receiving phrenic nerve stimulation for diaphragmatic inspiration, stimulation of the extradiaphragmatic muscles could be coordinated with the diaphragm. Monitoring during upper thorax stimulation should include EKG recording to assess the occurrence of heart arrhythmia, which, if observed, would mandate stopping stimulation.

This new stimulator has important features for animal and early clinical testing. The companion report from these animals demonstrated effective implant intramuscular electrodes in extradiaphragmatic muscles for respiratory responses.²⁷ Thus, this platform works equally well with surface and implanted electrodes, and applications can be considered for both types of electrodes as well as progression from one type of electrode to the other. This technology is also being developed for other SCI applications including urinary control and prevention of skeletal-muscle disuse atrophy with avoidance of pressure ulcers.^{25,26}

Conclusions

Studies in an anesthetized and respiratory apneic adult canine model identified optimal surface stimulation methods of upper thorax and abdominal muscles to induce sufficient volumes for ventilation and cough. Optimized methods for abdominal stimulation included three sets of bilateral electrodes located 4.5 cm dorsal to the lateral line at the 8th interspaces to caudal to the 13th rib, 80 or 100 mA current, and 50 Hz frequency. Optimized upper thoracic stimulation methods with not more than slight-to-moderate forelimb movement included a single bilateral set of electrodes located over the 2nd intercostal space, 60 to 80 mA and 50 Hz. Exploratory results for combined muscle stimulation further increased the ventilatory volume and a model of glottal closure demonstrated increased air flow. Further study of this assistive technology is warranted for the goal of providing respiratory assistance to individuals with SCI.

Acknowledgement

This investigation was based upon work supported by the Office of Research and Development, the Rehabilitation Research and Development Service of the Department of Veterans Affairs (Grant B0348). Synapse Biomedical Inc. provided support including development and manufacturing of the 12-Channel Neuroprosthetic Platform and electrodes.

ORCID

Scott Sayers <http://orcid.org/0000-0002-1105-7905>

Kiratipath Iamsakul <http://orcid.org/0000-0003-4125-8908>

References

- Laghi F, Tobin MJ. Disorders of the respiratory muscles. *AM J Respir Crit Care Med* 2003;168(1):10–48.
- Bauman WA, Korsten MA, Radulovic M, Schilero GJ, Wecht JM, Spungen AM. Secondary medical consequences of spinal cord injury. *Top Spinal Cord Inj Rehabil* 2012;18(4):354–78.
- Wong SL, Shem K, Crew J. Specialized respiratory management for acute cervical spinal cord injury: a retrospective analysis. *Top Spinal Cord Inj Rehabil* 2012;18(4):283–90.
- Bach JR, Saporito LR, Shah HR, Singuee D. Decanulation of patients with severe respiratory muscle insufficiency: efficacy of mechanical insufflation-exsufflation. *J Rehabil Med* 2014;46(10):1037–41.
- Jarosz R, Littlepage MM, Creasey G, McKenna SL. Functional electrical stimulation in spinal cord injury respiratory care. *Top Spinal Cord Inj Rehabil* 2012;18(4):315–21.
- Onders RP, Elmo MJ, Khansarinia S, Bowman B, Dunkin B, Ingrasson PE, et al. Complete worldwide operative experience in laparoscopic diaphragm pacing: results and differences in spinal cord injured patients and amyotrophic lateral sclerosis patients. *Surg Endosc* 2009;23(7):1433–40.
- DiMarco AF, Ramond P, Onders RP, Ignagni A, Kowalski KE, Mortimer JT. Phrenic nerve pacing via intramuscular diaphragm electrodes in tetraplegic subjects. *Chest* 2005;127(2):671–8.
- DiMarco AF, Supinski GS, Petro JA, Takaoka Y. Evaluation of thoracic pacing to provide artificial ventilation in quadriplegics. *Am J Respir Crit Care Med* 1994;150(4):934–40.
- DiMarco AF, Takaoka Y, Krzysztof E, Kowalski E. Combined thoracic and diaphragm pacing to provide artificial ventilation in patients with tetraplegia. *Arch Phys Med Rehabil* 2005;86(6):1200–07.
- DiMarco AF, Supinski GS, Petro JA, Takaoka Y. Evaluation of intercostal pacing to provide artificial ventilation in quadriplegics. *Am J Respir Crit Care Med* 1994;150(4):934–40.
- Walter JS, Thomas D, Sayers S, Anthony APT, Crish T, Singh S. Respiratory Responses to Stimulation of Abdominal and Upper thorax Intercostal Muscles Using Multiple Permaloc® Electrodes. *J Rehab Research Dev* 2015;52(1):85–96.
- Ho CH, Triolo RJ, Elias AL, Kilgore KL, DiMarco AF, et al. Functional electrical stimulation and spinal cord injury. *Phys Med Rehabil Clin N Am* 2014;25(3):631–54.
- DiMarco AF, Kowalski KE. Activation of inspiratory muscles via spinal cord stimulation. *Respir Physiol Neurobiol* 2013;189(2):1010–6.
- Zhang X, Plow E, Ranganathan V, Huang H, Schmitt M, Nemunaitis G, et al. Functional magnetic stimulation of inspiratory and expiratory muscles in subjects with tetraplegia. *Phy Med Rehab* 2016;8(7):651–9.
- McCaughy EJ, Berry HR, McLean AN, Allan DB, Gollee H. Abdominal functional electrical stimulation to assist ventilator weaning in acute tetraplegia: A Cohort Study. *PLoS One* 2015;10(6).
- DiMarco AF, Kowalski KE, Hromyak DR, Geertman RT. Long-term follow-up of spinal cord stimulation to restore cough in subjects with spinal cord injury. *J Spinal Cord Med* 2014;37(4):380–8.
- Kowalski KE, Romaniuk JR, Brose S, Richmond MA, Kowalski T, DiMarco AF. High frequency spinal cord stimulation: a new method to restore cough. *Respir Physiol Neurobiol* 2016;232:54–6.
- Geertman RT, Hromyak DR, Frost FS, Creasey GH, Nemunaitis GA. Lower thoracic spinal cord stimulation to restore cough in patients with spinal cord injury: results of a National Institutes of Health-Sponsored clinical trial. Part II: clinical outcomes. *Arch Phys Med Rehabil* 2009;90(5):726–32.
- Lim J, Gorman RB, Saboisky JP, Gandevia SC, Butler J. Optimal electrode placement for noninvasive electrical stimulation of human abdominal muscles. *J Appl Physiol* 2007;102(4):1612–7.
- Taylor PN, Tromans AM, Harris KR, Swain ID. Electrical stimulation of abdominal muscles for control of blood pressure and augmentation of cough in a C3/4 level tetraplegic. *Spinal Cord* 2002;40(1):34–6.
- Hascakova-Bartova R, Dinant J-F, Parent A, Ventura M. Neuromuscular electrical stimulation of complete paralyzed abdominal muscles in spinal cord-injured patients: a pilot study. *Spinal Cord* 2008;46(6):445–50.
- Lee BB, Boswell-Ruys C, Butler JE, Gandevia SC. Surface functional electrical stimulation of the abdominal muscles to enhance cough and assist tracheostomy decannulation after high-level spinal cord injury. *J Spinal Cord Med* 2008;31(1):78–82.
- McCaughy EJ, Borotkancis RJ, Glee H, Folz RJ, McLachlan AJ. Abdominal functional electrical stimulation to improve respiratory

- function after spinal cord injury: a systematic review and meta-analysis. *Spinal Cord* 2016;54(9):628–639.
- 24 Lin V, Deng X, Xiong X, Lee Y, Sasee S, Hsiao I. Expiratory muscle activation using BION microstimulators in dogs. *J Spinal Cord Med* 2000;28(4):370–7.
- 25 Walter J, Wheeler JS, Bresler L, Sayers JS, Singh S. Neuroprosthetics for SCI bladder management: the argument for direct bladder stimulation. *Int J Rehabil* 2014;2:230.
- 26 Walter JS, Posluszny J, Wheeler J, Bresler L, Dieter R, Dieter RS, et al. Update on a 12-Channel, Laboratory, Neuroprosthetic platform for SCI: respiration, urinary, and muscle disuse atrophy applications. *Internal Med Rev* 2016;1–21; doi:10.18103/imr.v2i8.147.
- 27 Singh S, Walter JS, Dieter R, Sayers S, Kiratipath I, Thomas D, et al. Respiratory stimulation for cough using a 12-Channel neuroprosthetic platform with intramuscular mapping and Permaloc® electrodes: acute studies in adult canines. *Internal Med Rev* 2016;2(11).
- 28 Walter JS, Wurster RD, Zhu Q, Laghi F. Respiratory muscle pacing with chronically implanted intramuscular permaloc® electrodes: a feasibility study. *J Rehab Res Dev* 2011;48(2):103–14.
- 29 Walter JS, Wurster RD, Zhu Q, Staunton C, Laghi F. Stimulating multiple respiratory muscles with intramuscular Permaloc® electrodes. *J Spinal Cord Med* 2010;33:135–43.
- 30 Walter JS, Dunn R, Wurster RD, Laghi F. Microstimulators and intramuscular hook electrodes for the stimulation of Respiratory Muscles. *J Spinal Cord Med* 2007;30(4):338–45.
- 31 Dunn RB, Walter JS, Walsh J. Diaphragm and accessory respiratory muscle stimulation using intramuscular electrodes. *Arch Phys Med Rehabil* 1995;76(3):266–71.