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Implanted Phrenic Stimulation Impairs Local Diaphragm Myofiber Reinnervation in Amyotrophic Lateral Sclerosis

Diaphragm pacing through laparoscopically implanted intramuscular electrodes led to significant excess mortality in two recent randomized controlled trials in amyotrophic lateral sclerosis (ALS), DiPALS (Diaphragm Pacing in patients with Amyotrophic Lateral Sclerosis) (1) and RespiStimALS (Early Diaphragm Pacing in Patients with Amyotrophic Lateral Sclerosis) (2). In the RespiStimALS study, the decrease in survival observed with active stimulation was associated with an accelerated deterioration of lung function (2). The underlying mechanisms were not addressed by the original RespiStimALS publication (2). With the pacing technology used in RespiStimALS (intradaphragmatic phrenic stimulation, NeurRx DPS; Synapse Biomedical), termination of the phrenic nerves is stimulated by the diffusion of electrical current through the adjacent diaphragm muscle mass. Animal studies have shown that muscle electrical activation after experimental nerve injury can impair reinnervation and exacerbate atrophy (3, 4). In the present ancillary analysis of the RespiStimALS study, we used histopathological and electrophysiological data to test the hypothesis that diaphragm

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Author Contributions: Study concept and design: T.S. and G.B. Data acquisition and analysis: all authors. Drafting of the manuscript or figures: R.G.-C., I.R., T.S., and G.B. All of the authors critically reviewed and approved the final version of the manuscript.

Table 1. Clinical Characteristics and Fiber-Type Grouping at Baseline

	Active Stimulation (n = 19)				Sham Stimulation (n = 20)				Statistics*
	n (%)	Mean	Median	Range	n (%)	Mean	Median	Range	
Age, yr	—	56	57	32–78	—	52	53	26–70	NS
Sex, M	16 (84)	—	—	—	7 (35)	—	—	—	P = 0.003
Bulbar onset	1 (5)	—	—	—	6 (30)	—	—	—	NS
Disease duration at baseline, mean, mo	—	28	23	9–56	—	32	25	14–108	NS
FVC% predicted at baseline [†]	—	87	88	47–113	—	82	79	65–114	NS
ALSFRS-R score at baseline	—	31	34	20–42	—	32	33	19–45	NS
Type I grouped myofibers [‡] , %	—	8.6	5.1	0.2–35.6	—	8	6.1	0–35.5	NS
Type II grouped myofibers [‡] , %	—	2	1.3	0–8.9	—	1.9	0.7	0–12.5	NS

Definition of abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised, score 0 (total disability) to 48 (no disability); NS = not significant.

*Mann-Whitney *U* tests and Fisher's exact tests were used to compare continuous data and categorical data, respectively.

[†]Preoperative measurement of FVC was performed immediately (1–19 d) before surgery.

[‡]Fiber-type grouping was manually assessed by determining the number of fibers that were surrounded on all sides by myofibers of the same type in each section (7). The number of fibers inside each cluster of grouped myofibers was counted in each section. The total number of enclosed fibers, for slow- and fast-twitch myofibers, was then calculated for each patient and expressed as a percentage of the overall number of myofibers of the same type. The values presented here are the mean, median, and range of the percentage of enclosed myofibers, in the active- and sham-stimulation groups.

spacing could have negatively influenced the development and/or maintenance of diaphragm reinnervation, which is a crucial compensatory mechanism in patients with ALS (5).

Methods

The patients described in this study gave written consent to participate in a diaphragm biopsy substudy of the RespiStimALS protocol (http://www.lppr.org/RespiStimALS/RespiStimALS_Protocol.pdf), which was approved by the local ethics committee (6). All of the patients were treated with riluzole. Adequate samples were obtained

from the left costal diaphragm in 39 patients (active stimulation, *n* = 19; sham stimulation, *n* = 20; Table 1) at the end of the surgical procedure to implant stimulation electrodes (2). The histological methods used have been described in detail elsewhere (6). Briefly, the muscle fiber type was established by immunofluorescent staining for myosin heavy chain isoforms, using 8- μ m-thick cryostat muscle sections. For each sample, 400–6,000 fibers were analyzed (mean, 2,037). Reinnervation-induced fiber-type grouping was manually assessed by determining the number of fibers that were totally enclosed by myofibers of the same type in each section (7).

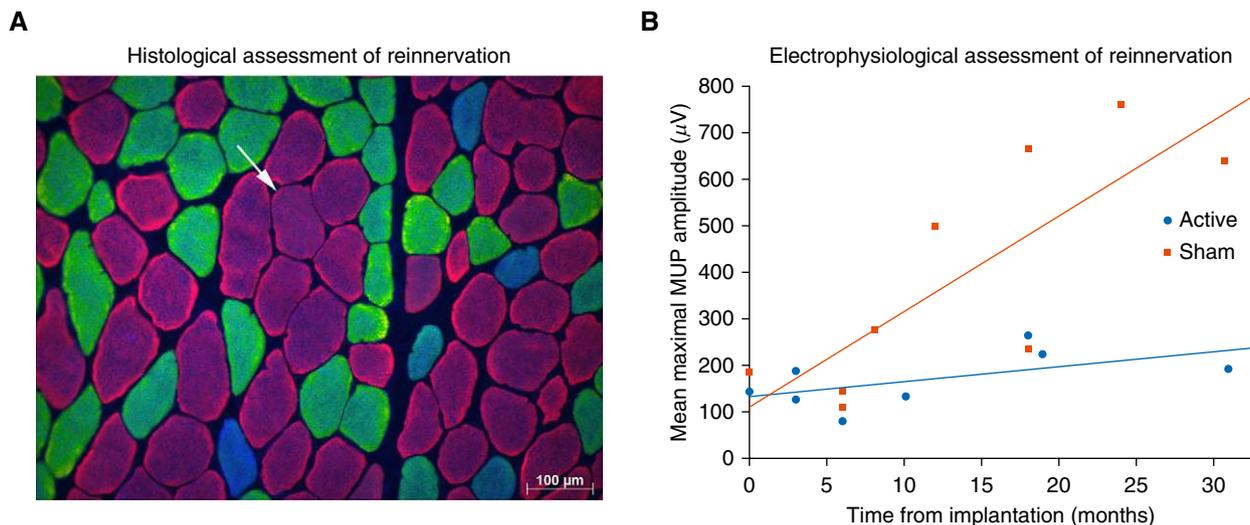


Figure 1. Diaphragm reinnervation depending on the stimulation group. (A) Histological assessment of reinnervation at baseline. A cross-section of a diaphragm muscle was observed under a light microscope after immunohistochemistry was performed using antibodies against major histocompatibility complex type I (slow-twitch myofibers, in red) and against MHC (myosin heavy chain) IIa and IIx (fast-twitch myofibers, in green and blue, respectively). Fiber-type grouping, reflecting muscle reinnervation, was manually quantified by determining the number of fibers enclosed by fibers of the same histochemical type in each muscle section. The arrow indicates an enclosed slow-twitch myofiber. Fiber-type grouping was quantitatively similar between the two stimulation groups at implantation. Scale bar, 100 μ m. (B) Electrophysiological evaluation of reinnervation in active- and sham-stimulated patients with amyotrophic lateral sclerosis. In patients in the sham-stimulation group, the later the recordings were performed after implantation, the higher were the amplitudes of the maximal motor unit potentials (MUPs) obtained from the intradiaphragmatic electrodes. This was not the case for patients in the active-stimulation group. Although the intercepts did not differ, the slopes of the time–MUP size relationships were significantly different between the two groups, with a significantly steeper slope of the time–MUP amplitude relationship ($\times 6.4$) in the sham-stimulation group ($P = 0.013$).

Table 2. Intradiaphragmatic Recordings of the Left Hemidiaphragm Electrical Activity during Spontaneous Breathing

Stimulation Group	Patient	Time from Implantation (mo)	Mean Maximal MUP Amplitude (μV)
Active	001-0007	31	192.2
	001-0014	18	263.8
	001-0021*	28	683.5
	001-0033	19	223.6
	001-0041	0	143.0
	001-0043	3	126.5
	001-0045	10	132.7
	001-0046	6	79.9
	001-0056	3	187.6
Sham	001-0009	24	759.7
	001-0010 [†]	24	621.1
	001-0010 [†]	31	639.4
	001-0023	18	665.0
	001-0048	6	144.4
	001-0050	8	276.5
	001-0051 [‡]	7	173.6
	003-0002	0	185.0
	016-0001 [†]	6	488.2
	016-0001 [†]	12	498.8
	017-0001	18	235.8
	017-0003	6	109.3

Definition of abbreviation: MUP = motor unit potential.

*Patient 001-0021 experienced diaphragm pacing-related pain requiring an important reduction in pulse intensity in the left side. For the duration of the study, he only tolerated very low pulse intensities that were not sufficient to achieve effective diaphragm contractions and thus did not receive "active" stimulation. He was therefore excluded from analysis.

[†]Patients 001-0010 and 016-0001 were recorded twice, at 24 and 31 months, and 6 and 12 months, respectively, after implantation. Only the latest value was used for statistical analysis.

[‡]For patient 001-0051, criteria for initiating noninvasive ventilation were met 3 months after implantation and the patient was subsequently switched to active stimulation for the rest of the study, in line with the RespiStimALS protocol.

Morphometric analyses were performed in a blinded manner regarding the stimulation group.

In 19 of these patients, intradiaphragmatic electromyographic recordings were performed using the implanted stimulation electrodes from 0 to 31 months after implantation (active stimulation, $n=9$; sham stimulation, $n=10$), as planned in the RespiStimALS protocol. The analysis was restricted to the left side because the biopsies were systematically taken from this hemidiaphragm. The electrical activity of the left hemidiaphragm during spontaneous breathing was digitized at 10 kHz and rectified (Power 1401; Cambridge Electronic Design). The maximal amplitude of motor unit potentials (MUPs) during rhythmic bursts (21–121 bursts per patient; mean, 45), which is related to the number of myofibers in the corresponding motor unit territory, was determined by offline analysis (Signal 5.05a; Cambridge Electronic Design).

Statistical analyses were performed with XLSTATS 2017 (Addinsoft) and the MATLAB R2017b environment (The MathWorks Inc.). Mann-Whitney U tests and Fisher's exact tests were used to compare continuous data and categorical data, respectively. An affine relationship between time from implantation and maximal MUP

amplitude was first evaluated in each group separately using ordinary least squares. The corresponding residuals were Gaussian (Shapiro-Wilk test), and the variances were significantly different ($s^2_{\text{Sham}}/s^2_{\text{Active}}=9.2$, $P=0.015$, Fisher's test). Then, the following model was used to model the data simultaneously in the two groups: $y^k = \theta_1 + \theta_2 x^k + \theta_3 c^k + \theta_4 c^k x^k + w^k$, where k indexes the patient, y^k denotes the maximal MUP amplitude, x^k is the time from implantation, c^k is the group ($c^k=0$ for the active group, and $c^k=1$ for the sham group), and w^k is a zero-mean Gaussian variable. With this global model, testing the equality of the slopes amounts to testing the nullity of parameter θ_4 , and testing the equality of the intercepts amounts to testing the nullity of parameter θ_3 . Because the variances in the two groups were significantly different, the estimation and tests were performed using weighted least squares, with the weights being taken equal to the inverse variance estimates s^2_{Sham} and s^2_{Active} obtained from the first separate regressions using ordinary least squares. All statistics were two tailed, and the level of significance was set at 0.05.

Results

The clinical and histological characteristics of active- and sham-stimulated patients were similar at implantation (6), except for a significant overrepresentation of men in the active-stimulation group (Table 1). In particular, there was no difference in the proportion of grouped myofibers (Table 1 and Figure 1A), the histological hallmark of repeating cycles of denervation and reinnervation (7).

Left diaphragm electrical activity was recorded for 19 of 39 patients (Table 2). In two of the patients, two recordings were obtained (at 24 and 31 mo, and 6 and 12 mo, respectively), but only the last recording was used for analysis. One patient was excluded from analysis because, due to stimulation-related pain, he could not tolerate a sufficient pulse intensity to achieve effective diaphragm contractions. Another patient, who was randomized to sham stimulation, was excluded from analysis because he was placed under noninvasive ventilation after only 3 months and therefore switched to active stimulation according to the protocol (2). As a result, the analysis of the electrophysiological recordings pertained to 17 patients (active stimulation $n=8$, sham stimulation $n=9$). In the sham-stimulation patients, the later the recordings were performed in relation to the implantation, the higher were the maximal MUP amplitudes (Figure 1B). This was not observed in the active-stimulation patients. Indeed, although the intercepts were not significantly different, the slope of the relationship between maximal MUP amplitude and time was roughly 6.4 times steeper with sham stimulation than with active stimulation ($P=0.013$).

Discussion

Reinnervation of denervated muscle fibers is a crucial compensatory mechanism (5) in patients with ALS, and motor function is preserved as long as it compensates for motor neuron degeneration, as observed in long-term survivors (8). At the time of the biopsy, fiber-type grouping, reflecting the level of reinnervation in the diaphragm at implantation, was quantitatively similar between the two stimulation groups. In ALS, efficient reinnervation leads to motor unit enlargement, with an increase in the muscle fiber content of surviving units (5). Because the MUP amplitude reflects the motor unit size, the absence of an increase in MUP amplitude as a function of time in the active-stimulation group supports the notion of a pacing-induced defective reinnervation.

The use of electrical stimulation to treat denervated muscles is controversial. In the context of acute nerve injury with immediate surgical

repair (9), electrical stimulation can improve reinnervation and functional recovery. On the contrary, electrical stimulation applied after partial denervation has been shown to dramatically reduce terminal sprouting (3). Electrical stimulation therefore delays reinnervation and impairs functional recovery of partially denervated muscles (10). In the ALS context of successive cycles of partial denervation followed by reinnervation by the surviving motor neurons (11), diaphragm electrical stimulation could thus have impaired compensatory reinnervation, leading to faster deterioration of respiratory function and reduced survival. Of note, conditions in which diaphragm pacing is used successfully (i.e., high-level spinal cord lesions and central hypoventilation [12]) are characterized by the absence of phrenic denervation.

Conclusions. This study provides the first mechanistic explanation for the harmful effect of diaphragm pacing in patients with ALS by showing impaired compensatory reinnervation in patients receiving active stimulation. ■

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