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Multivariate assessment of differences between a neuromuscular electrical stimulation therapy and robotic treadmill training in the rehabilitation of spinal cord injured rats

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***Abstract*— A study was conducted to evaluate and compare the effects of two different rehabilitation therapies on spinal cord injured (SCI) rats: neuromuscular electrical stimulation which is timed to robotic treadmill training (NMES+RTT) and RTT alone. Several electromyography (EMG) based variables were measured, but most did not change significantly after treatment, contrary to observations of overall qualitative stepping ability. However, when the variables are viewed in multi-dimensional space, there are visible differences between changes after NMES+RTT vs. those after RTT only. Principal component analysis (PCA) and k-means clustering were applied to the multivariate data. The data in principal component space was significantly separated, according to the Euclidean distance. PCA also provided a straightforward tool for selecting which combination of measures to compare. The measures which best separated out the differences between NMES+RTT and RTT were percentage of steps associated with bursts, burst-to-step latency, and the standard deviation of this latency, even though these measures did not always show the greatest statistical significance individually. Thus, the rehabilitative effects of NMES+RTT are not necessarily reflected in individual EMG measures, but rather in a combination of the measures representing a multi-dimensional space.**

I. Introduction

It is estimated that in the United States there are approximately 12,000 new spinal cord injuries (SCI) per year [1]. SCIs can have a devastating effect on multiple aspects of quality of life, ranging from mobility to mental health [2][3]. That is why it is important to research therapies that can provide rehabilitation for SCI patients. The ability to characterize data in a meaningful way is important so that the efficacy of therapies can be assessed and further developed. In this study, two therapies were compared: robotic treadmill training (RTT) only, which is becoming an increasingly common rehabilitation therapy for walking after SCI [4][5], and an investigational therapy in which neuromuscular electrical stimulation (NMES) is used in conjunction with RTT therapy [4][6][7]. NMES+RTT was observed to influence the EMG activation profile during stepping in a previous study [8] but has not been compared with RTT only. In the present study, we computed the EMG profile and other EMG-based measures that could potentially be influenced by the two therapies. When developing an investigational therapy such as NMES+RTT, comprehensive analysis of the data i the chances of understanding its effects and underlying mechanism. Thus, a multidimensional analysis tool, namely PCA, was applied to the data to determine the differences in effect of NMES+RTT and RTT.

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II. Methods

A. Experimental design

Twenty rats were spinally contused at the T9, mid-thoracic level, utilizing a force impactor (Precision Systems & Instrumentation, Lexington, KY) in order to induce incomplete spinal cord injury and paraplegia. After two weeks of recovery they were bilaterally implanted with a pair of stimulating and EMG recording wire electrodes each in the *tibialis anterior* (TA) muscle. The rats were then separated into two groups. Group 1 consisted of 7 subjects that received RTT only for the first two weeks of training and NMES+RTT for the subsequent two weeks of training. Group 2 consisted of 6 subjects that received NMES+RTT for the first two weeks of training and RTT only for the subsequent two weeks of training. The remaining 7 out of 20 rats were unable to complete the training and were thus excluded from the study. The rats were tested at baseline, after two weeks, and again at the end of the four weeks (B, 2wk, F, respectively).

B. RTT and NMES+RTT

In both RTT and NMES+RTT therapies, the rats were secured in a vest above the treadmill that provided 85% body weight support during both training and testing sessions. During RTT, robot arms were strapped to the rat's ankles to guide stepping. Both groups utilized the same protocols for RTT, but NMES+RTT also incorporated biphasic stimulation during the first 50% of the swing phase if the rat's hindlimb was sufficiently tracking the pre-programmed trajectory [7]. Stimulation pulse parameters were set to 70 pulses per second (pps) at a 100 μs pulse width, and at 1.5 times the motor threshold based loosely on work by a group similarly applying NMES to rodent hindlimb after spinal contusion.

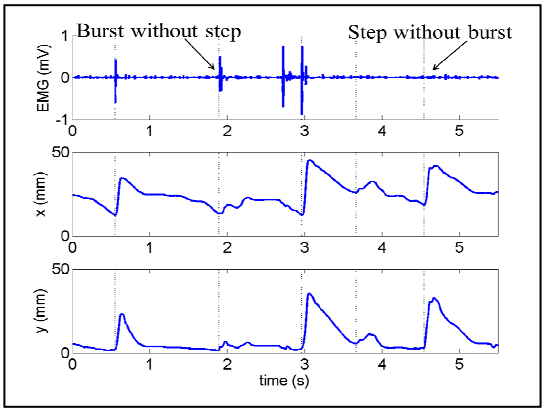

Figure 1. Simultaneously recorded ankle flexor EMG and *x* and *y* position of the ankle during testing. Normally bursts are closely associated in time with detected steps (vertical dashed lines). However, in poorly stepping animals, sometimes a burst occurs without a step within a few hundred milliseconds, and vice-versa.

C. EMG analysis

During testing, EMG and hindlimb position were recorded while the rat performed treadmill stepping at 85% body weight support with no stimulation and no robotic assistance. Ten measures were computed from the recorded EMG based on the hypothesis that NMES+RTT would improve neuromuscular control of stepping via changes in the spinal cord (Table I).

TABLE I. SYMBOL LEGEND OF COMPUTED MEASURES

Symbol	Measure
τ	Burst-to-step latency
σ_τ	Standard deviation of burst-to-step latency
%BwS	Percentage of bursts associated with steps
%SwB	Percentage of steps associated with bursts
A_B:A_S	Burst:step iEMG amplitude ratio
Δ_B: Δ_S	Burst:step duration ratio
γ	Concentration of energy in EMG profile
μ	Location of peak center in EMG profile
σ	Width of peak in EMG profile
A	Amplitude of peak in EMG profile

EMG burst and step detection methods are described in [8], but also briefly recapitulated here. EMG bursts were detected by thresholding the envelope, which was obtained by rectifying and low-pass filtering the raw EMG signal. Steps were detected by finding local minima in the *x* position of the hindlimbs, obtained by optical sensors in the robot. These steps were validated by checking that the *x* displacement exceeded a minimum of 5 mm. Burst-to-step latency, τ, measures the time between the start of an EMG burst and the nearest valid step. If latencies were greater than 200 ms, they were considered not associated with that step (Fig.1). Further definition of %BwS and %SwB is given in [9]. The amplitude of EMG was measured by integrating the EMG envelope (iEMG) during a burst and during a step, and then taking the ratio. The same definition as used in [8] was applied here to compute the EMG profile. γ measures the concentration of energy in the EMG activity during roughly the same period during which stimulation would have been applied during NMES+RTT training. A Gaussian curve (Eq. 1) was fit to the EMG profile of each rat to parameterize the center and width of the peak.

$$S(g) = \frac{1}{\sqrt{2\pi}\,\sigma} e^{-\frac{1}{2}\left(\frac{g-\mu}{\sigma}\right)^2} \qquad (1)$$

D. Statistical significance testing on individual EMG variables

Paired t-tests were conducted on each of the ten variables to compare the effect of NMES+RTT with that of RTT only. First, changes in each of the measures from baseline to 2-week testing and from 2-week to 4-week testing were computed. Then, differences were computed between the changes proceeding NMES+RTT therapy with those proceeding RTT only therapy. In other words, if x_{tp} represents one of the EMG variables measured at a given test-point *tp* (0, 2, or 4-week), $x_2 - x_0$ represents the changes in measure x after RTT only, and $x_4 - x_2$ represents the change after NMES+RTT. Table II displays the difference in effect of NMES+RTT relative to RTT; i.e., $(x_4 - x_2) - (x_2 - x_0)$ for Group 1 and $(x_2 - x_0) - (x_4 - x_2)$ for Group 2. The *p* values resulting from the paired t-tests are listed in the shaded rows below each of the differences. The two groups were kept separate to determine if the order in which therapies were administered made a difference. The dominant (D) and non-dominant (ND) hindlimbs were also analyzed independently, since they are controlled with semi-independent spinal and neuromuscular circuitry, and were observed to show differences in stepping ability for each individual rat. A Gaussian curve (Eq. 1) was fit to the EMG profile of each rat to parameterize the center and width of the peak.

E. Multivariate analysis

We computed measures which characterize the EMG burst activity, but based on the changes in these individual measures after NMES+RTT vs. RTT, it was unclear whether the two therapies had significantly different effects. Thus, we computed the principal components (PC) of the 10-dimensional data set using MATLAB's built-in `princomp` function (The MathWorks, Inc., Natick, MA). The resulting PCs were interpreted as representations of the data in a lower-dimensional vector space, which allowed the effects of the two therapies to be more easily compared. The ratio of the cumulative sum of the eigenvalues associated with the PCs, rank-ordered by eigenvalue, and the total sum of eigenvalues was calculated and a threshold of 0.99 was set on the ratio to determine N_{PC}, the number of PCs required to comprise 99% of the variance of the signal. The data was projected onto the 1st N_{PC} PCs and plotted to visualize any separation between the effects of NMES+RTT and RTT (Fig.2). The changes after RTT only and after NMES+RTT were classified into two separate "clusters". The intra-cluster (Eq. 2) and inter-cluster (Eq. 3) distances were calculated as follows:

$$D(a_i, \bar{a}) = \left[\sum_{j=1}^{N_{PC}} \left(a_{i,j} - \bar{a}_j\right)^2\right]^{1/2} \qquad (2)$$

$$D(a_i, \bar{b}) = \left[\sum_{j=1}^{N_{PC}} (a_{i,j} - \bar{b}_j)^2\right]^{1/2} \quad (3)$$

where a_i represents the i^{th} multivariate data point; $a_{i,j}$ represents the j^{th} coordinate, or dimension, of a_i; and $\bar{a}$ represents the centroid of all of the data points a_i in cluster a, such that $\bar{a}_j = \langle a_{i,j} \rangle$. Similar definitions apply for all data points in cluster b.

The differences between the intra-cluster and inter-cluster distances were tested for statistical significance using a paired t-test. As a control, data points were randomly designated into two clusters, and the intra-cluster and inter-cluster distances were also compared using a paired t-test. If the separation between two clusters distinguished by treatment can be used to show true differences in effect, then the intra- and inter-cluster distances are expected to be significantly different when the data points are separated by treatment but not when separated into random clusters.

PCA was then used to select which measures to assess in order to determine *how* the therapies affected stepping capability. The weights of those first N_{PC} vectors indicate how much each of the EMG variables contributes to the given PC. The absolute value of the weights in each of the first N_{PC} vectors were ranked in descending order, and the N_{PC} measures with the lowest sum of ranks were selected. These N_{PC} measures were then plotted as multidimensional vectors to view how the therapies affected stepping ability (Fig. 3).

III. Results

A. Comparing the effect of NMES+RTT vs. RTT on individual measures

Results of the paired t-tests on individual measures, as seen in Table II, did not yield any obvious significant indicators of improvements in stepping ability across all groups. Few significant p values were distributed across different measures, making it difficult to conclude that any one of them could be consistently used as a reliable statistic.

B. Principal component analysis

In all cases, three PC vectors were needed to capture 99% of the variability in the data; i.e., $N_{PC} = 3$. When viewed in principal component space, a differential effect of the two therapies could be visually detected (Fig. 2). A comparison of the intra-cluster distances with the inter-cluster differences also revealed that NMES+RTT and RTT only had significantly different effects on the 10 EMG variables that were measured (Table 3). The intra-cluster distances were significantly different from the inter-cluster distances when the data were clustered by treatment, but not significantly different when randomly separated.

TABLE II. Differences between NMES+RTT vs. RTT and significance

Group / Side	%SwB	%BwS	τ	σ_τ	Δ_B: Δ_S	A_B:A_S	γ	μ	σ	A
GROUP1 / ND	-20.9	-14.6	-63.1	81.2	-0.03	-0.10	8.4	0.04	0.17	-0.06
p	.07	.22	.04	.06	.35	.25	.15	.10	.02	.31
GROUP1 / D	39.4	8.2	23.7	103.7	-0.06	0.04	3.3	0.04	-0.02	-0.02
p	.00	.28	.30	.04	.25	.37	.37	.19	.45	.39
GROUP2 / ND	85.1	-32.6	-4.2	94.0	-0.19	0.26	0.7	-0.01	0.10	-0.02
p	.03	.14	.41	.16	.02	.08	.08	.47	.34	.38
GROUP2 / D	-24.0	-2.8	39.1	-53.8	0.10	-0.14	11.3	-0.03	0.23	-0.19
p	.22	.46	.24	.05	.33	.37	.37	.33	.13	.01
Direction of Improvement	↑	↑	↓	↓	↓	↑	↑	↓	↓	↑

TABLE III. Inter vs. intra cluster distances in principle component space and significance

	after RTT only		after NMES+RTT	
	$D(x_i, y_C) - D(x_i, x_C)$	*p*	$D(x_i, y_C) - D(x_i, x_C)$	*p*
G1-D	38.2	.08	71.7	<<.01
G1-D-Rand	-4.9	.76	6.9	.36
G1-ND	23.3	.15	69.2	<<.01
G1-ND-Rand	19.2	.10	-9.8	.74
G2-D	24.7	.14	27.5	0.017
G2-D-Rand	3.6	.22	5.4	.32
G2-ND	78	.01	59.2	.07
G2-ND-rand	3.9	.34	1.9	.69

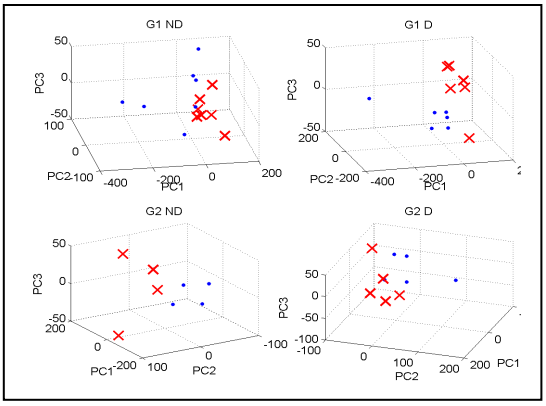

Figure 2. Changes in EMG measures in principal component space. Projections onto the first 3 components show separation between the two clusters, representing changes after RTT only (•) vs NMES+RTT (x) therapy.

C. Relating PC back to physiological EMG variables

The clear segregation of data in principal component space revealed the existence of a differential effect by NMES+RTT relative to RTT only. In order to investigate how the stepping changed in terms of physiological quantities and determine whether NMES+RTT or RTT helped to improve stepping, EMG measures that had the greatest

contribution to the first N_{PC} principal components were viewed in 3-space (Fig. 3). The percentage of steps associated with bursts (%SwB) was a major contributor in all cases. In most cases, burst-to-step latency (τ) and variability in burst-to-step latency (σ_τ) also made substantial contributions to the selected PCs. The differences in effect between NMES+RTT and RTT varied depending on which group (NMES+RTT administered first or second) and which side (dominant or non-dominant). With the exception of Group 2 dominant side, the differential effect tended to be roughly the same; i.e., the direction of the lines within each subplot are generally the same. When RTT was administered first, NMES+RTT caused τ to decrease more than RTT only on the non-dominant side and led to greater increases in %SwB on the dominant side when NMES+RTT was administered first.

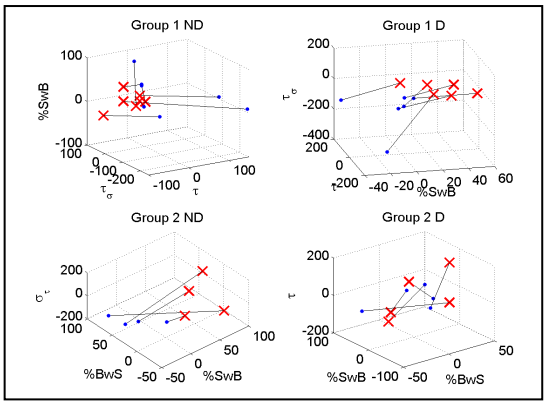

Figure 3. Comparison of changes in EMG variables after RTT only (•) and NMES+RTT (x) for each limb (ND – non-dominant, D – dominant side) and each group. Variables were selected according to their contribution to the 1st N_{PC} PC vectors. Data points corresponding to the same rat are connected by lines.

IV. Discussion

A. PCA as a selector for variables

By using PCA on EMG data collected after different SCI rehabilitation therapies, redundant information was effectively eliminated, which can otherwise clutter our understanding of how therapies affect stepping ability. PCA allowed us to define which combination of variables were most differentially affected by the two therapies of interest. PCA identifies linear correlations between variables, whereas non-linear correlation is likely to exist in the EMG data. Despite this, the EMG variables were linearly correlated enough that PCA was able to help identify the most influential EMG variable. The variables which carried the most weight on average in the most significant PC vectors were: τ, σ_τ, %SwB, and %BwS. This indicates that perhaps those measures are more informative indicators of progress from the EMG measures. The fact that the two groups clustered separately for those variables indicates that as a group, those combinations of variables are more significantly altered than any one EMG measure alone, and that those combinations might be of more interest when evaluating progress.

B. PCA as a tool for showing multivariate differences between treatments

PCA allows for clearer distinctions between therapies that were not made apparent using univariate statistical significance testing. It allows us to see the difference in effect each therapy has on co-varying variables. The weights of the original EMG variables in the most significant PCs serves as a tool for selecting which combinations of variables to analyze in order to compare the effectiveness. Quantitative analysis of the separation of clusters in PC space indicate that NMES+RTT does affect stepping differently than RTT only, but not necessarily in any individual EMG measure, rather in a combination of multiple measures. Depending on the dominance of the hindlimb and whether it was administered before or after RTT therapy, NMES+RTT appeared to decrease burst-to-step latency and increase percentage of steps associated with bursts more than RTT alone.

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