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RESEARCH

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Efficacy of high- versus moderate-intensity spatially distributed sequential stimulation in subjects with spinal cord injury: an isometric study

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Abstract

For producing isometric contractions, spatially distributed sequential stimulation (SDSS) has been demonstrated to be superior to conventional single electrode stimulation (SES) in terms of fatigue reduction and the power output produced by the muscle. However, the impact of stimulation parameters, particularly stimulation intensity, on the effectiveness of SDSS is not sufficiently understood. The aim of this work is to compare the fatigue-reducing capabilities of SDSS at two significantly different electrical stimulation intensities in individuals with lower-limb motor-complete spinal cord injuries, in order to understand the impact of stimulation intensity on the effectiveness of SDSS. Two experiments were conducted, focusing on isometric contractions of the quadriceps muscle group (Experiment 1) and the vastus lateralis muscle (Experiment 2). The effectiveness of high-intensity SDSS was compared to that of moderate-intensity SDSS, with SES serving as a reference. Seven subjects with spinal cord injuries participated in the study. Fatigue and force metrics, including time to fatigue (TTF) and force-time integral (FTI), were analyzed for both electrical stimulation intensity levels. Statistical analysis indicated that the advantages of SDSS over SES in reducing muscle fatigue and enhancing force generation were significantly diminished at high intensity compared to moderate intensity. These findings provide valuable scientific insights into the practical applications of SDSS and contribute to a deeper understanding of its mechanisms in mitigating muscle fatigue. Further research is recommended to explore the effects of various stimulation parameters to optimize SDSS for different muscle groups and functional tasks.

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Introduction

Transcutaneous neuromuscular electrical stimulation is a rehabilitative technique that uses surface electrodes to induce muscle contractions by generating action potentials in the axons of lower motor neurons innervating paralyzed muscles [1]. Functional electrical stimulation (FES) builds upon neuromuscular electrical stimulation by coordinating these muscle contractions in a sequenced manner to replicate functional movements [2, 3]. Cycling [4–6], walking [7, 8], and grasping [9] are among the most common applications of FES for individuals with upper motor neuron lesions, such as spinal cord injury (SCI) and stroke [10].

FES-based exercise has been shown in some studies to improve cardiovascular and respiratory functions [11–13], protect against and accelerate the healing of pressure sores by promoting muscle contractions that facilitate changes in body position [14], reduce the risk of osteoporosis and bone fractures [15, 16], and improved muscle strength and motor control [17, 18]. Nevertheless, it is important to note that the reported benefits of FES can vary depending on the specific protocol and population studied, with some studies reporting limited or no significant effects [19, 20].

However, FES recruits motor units based on their proximity to the stimulation site and in the reverse order of the natural recruitment pattern, leading to a rapid decline in the force produced by the stimulated muscles [21]. In contrast, voluntary muscle contractions recruit motor units based on their size, beginning with smaller axons that innervate slow, fatigue-resistant muscle fibers, and progressing to larger axons that innervate fast, easily fatigable muscle fibers [22]. Since the size of an axon determines its activation threshold, low-level electrical stimulation tends to activate larger axons first, resulting in an earlier onset of muscle fatigue [23]. Additionally, conventional electrical stimulation, known as single electrode stimulation (SES), employs relatively large transcutaneous electrodes (e.g., 5×9 cm² for quadriceps muscle group [5]) placed over multiple motor units, activating them with every stimulation pulse at high frequencies. This contrasts with voluntary contractions, where motor units are activated asynchronously and with lower frequencies, allowing for longer rest periods for each motor unit [21].

To reduce SES-induced muscle fatigue, the stimulation can be distributed across multiple smaller electrodes that cover different motor points, mimicking the natural pattern of voluntary contractions. Each electrode activates a different pool of motor units at a lower frequency than SES, while still maintaining a strong, fused muscle contraction. This approach has been shown to effectively reduce muscle fatigue when the electrodes are placed over different synergistic muscle bellies [24–27] or even over the same muscle belly [28–36]. The latter method, known as spatially distributed sequential stimulation (SDSS), uses four small, closely spaced electrodes placed over the same surface as the SES electrode. Each electrode stimulates at one-fourth of the typical SES frequency (10–15 Hz), with a 90° phase shift of pulses between electrodes.

Greater fatigue resistance of SDSS compared to SES has been demonstrated during isometric contractions of the quadriceps muscle group in subjects with SCI [33, 35, 37]. In all three studies, a similar electrode setup was used: a 2×2 matrix of 4.5×2.5 cm² electrodes placed over the entire quadriceps muscle group. Comparable results were obtained in studies where SDSS was applied to perform a dynamic knee extension task in both able-bodied [32] and SCI subjects [29]. In these cases, the 2×2 matrix of 4.5×2.5 cm² electrodes was positioned as close as possible to the motor points of the vastus medialis and vastus lateralis muscles. However, two recent studies [38, 39] did not observe a significant difference in the fatigue resistance of SDSS. One possible reason for this could be the higher intensity used during the experiments. Although the fatigue reduction of SDSS compared to SES has been demonstrated in paralyzed muscles with various electrode configurations, none of the studies have specifically investigated the effect of electrical charge magnitude. Charge can be modulated by changing pulse width or amplitude. One hypothesis is that the close spacing of electrodes in SDSS may lead to an overlap of electric fields as stimulation intensity increases, as discussed in [38]. This overlap could activate a larger number of muscle fibers more frequently, thereby diminishing the intended fatigue-reducing effect of SDSS. This phenomenon was also observed in a theoretical computational analysis of the electric fields generated by SDSS in the tibialis anterior muscle [40].

The present study examines the fatigue reduction of SDSS at high and moderate electrical stimulation intensities in subjects with motor-complete SCI. The effects of these stimulation strategies were evaluated during isometric contractions of the quadriceps muscle groups (Experiment 1) and the vastus lateralis muscles (Experiment 2). The primary goal was to compare the effectiveness of high-intensity SDSS with moderate-intensity SDSS, using SES as a reference. While deductions can be made, the experiments were not specifically designed to directly compare SDSS and SES. The outcomes of these experiments provide further insight into the practical applications of SDSS. Additionally, the study aims to contribute to a better understanding of the mechanisms underlying SDSS.

Methods

Subjects

Seven adult male subjects with lower-limb motor-complete spinal cord injuries participated in the study. All subjects met the inclusion criteria: a spinal cord injury spanning from cervical 4 to thoracic 12 spinal segments, no voluntary movement or pain sensation in the legs (ASIA A or B), and at least 12 months having elapsed since the injury. The exclusion criteria stipulated that the subjects must not have sustained lower limb fractures within the past year or have open wounds or rashes on the skin surface where the electrodes would be placed. Five subjects had previous experience with FES of the studied muscles, four of whom were undergoing FES-cycling strength training at the time of the study. To mitigate the effects of residual fatigue, all subjects were asked to refrain from using FES for at least 48 h before the experiments were conducted.

During the study, one subject (P6) was excluded during the second session of Experiment 1 (on the quadriceps muscle group) due to intense spasms that persisted despite being on antispasmodic medication (Baclofen). Efforts to repeat the session on another day yielded the same outcome, necessitating exclusion. Additionally, prior to Experiment 2 (on the vastus lateralis muscle), another subject (P7) declined to participate due to personal reasons. As a result, six participants were included in Experiment 1, and five participants were included in Experiment 2.

Before participating in the experiments, the subjects were provided with a detailed description of the study's purpose and testing procedures. Each subject provided written informed consent. The project was conducted in accordance with the Declaration of Helsinki, approved by the local Institutional Review Board, and registered at ClinicalTrials.gov (NCT06421753). Additional information about the subjects is provided in Table 1.

Instrumentation

Subjects were seated on a height-adjustable plinth with the back of the knee (popliteal fossa) in contact with the edge of the table and their thighs parallel to the ground. The knee joint angle was set to 90°, and a cushioned backrest was used to achieve a posterior inclination of 115°. In cases of insufficient trunk control, straps were used to stabilize the torso. The table height was adjusted so that the soles of the feet could not touch the ground.

An 8-channel MotiMove stimulator (3F-Fit Fabricando Faber, Belgrade, Serbia) [41], controlled with a custom-made LabVIEW program (National Instruments, Austin, TX, USA), was utilized to produce asymmetric balanced biphasic electrical pulses at a frequency of 48 Hz and a pulse width of 400 µs. To distribute pulses in SDSS mode, an anti-fatigue unit (AFU) device (3F-Fit Fabricando Faber, Belgrade, Serbia) was connected to the cathode of one of the stimulation channels. AFU distributes pulses sequentially from one input to four outputs with a 90° phase shift. The force produced by the stimulated muscle was measured with a force meter (Chronojump-Boscosystem, Barcelona, Spain) secured to the shank of the stimulated leg. The height of the mat table and the position of the sensor on the shank were measured to ensure identical experimental conditions between sessions. Force measurements were acquired with a frequency of 80 Hz and recorded in a text file for subsequent data analysis. An illustration of the experimental setup is shown in Fig. 1.

Electrode configuration

Two types of experiments with different electrode configurations were conducted on separate days to compare the fatigue reduction of SDSS at moderate and high electrical stimulation intensities. "Experiment 1" focused on the quadriceps muscle group, while "Experiment 2" was performed on the vastus lateralis muscle. In all experiments, four self-adhesive surface electrodes (Compex Dura-Stick plus) were placed distally (cathodes) while the reference electrode (anode) was placed proximally. This electrode configuration was selected based on literature protocols [42] and preliminary tests conducted during the study design phase. Before applying the electrodes, proximal and distal motor points of the rectus femoris (RF) and the vastus lateralis (VL) muscles were identified using anatomical landmarks as described in [43]. These motor points were marked with an indelible marker pen to serve as reference points for electrode placement. Prior to electrode placement, the thigh skin was sanitized with

Table 1 Study group demographics

Subjects	Age	Injury	ASIA	Time after spinal cord injury (years)	Previous FES experience	Current FES training
P1	46	C7 - C8	B	11	Yes	Yes
P2	30	C5 - C6	B	14	Yes	Yes
P3	59	T9 - T10	B	6	Yes	Yes
P4	59	C6 - C7	A	6	Yes	Yes
P5	62	C6 - C7	B	10	Yes	No
P6	49	T5 - T6	B	30	No	No
P7	38	C5 - C6	A	20	No	No

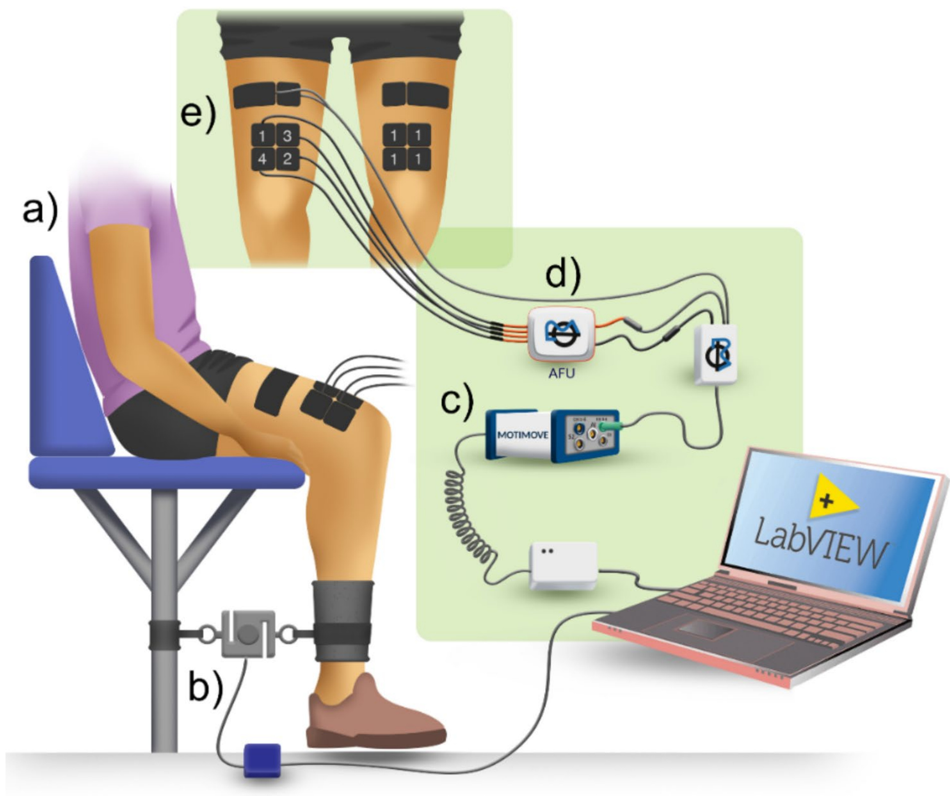

Fig. 1 Illustration of the experimental setup for performing isometric muscle contractions, used to record the generated force and assess the muscle fatigue using SDSS and SES electrode configurations. The picture illustrates: **a**) the sitting position, **b**) the force meter, **c**) the MotiMove stimulator with **d**) AFU device and **e**) the electrode configuration used in Experiment 1

an antiseptic spray to prevent skin irritation and ensure a clean surface.

In Experiment 1, the proximal electrode was made by connecting two electrodes to the anode of the same stimulation channel and thus creating an electrode with an effective surface area of 5×14 cm^2. Electrodes used for the anode were a 5×5 cm^2 electrode placed on the proximal motor point of the RF muscle and a 5×9 cm^2 electrode placed laterally, between the proximal motor points of the RF and VL muscles, just to the side of the 5×5 cm^2 electrode. Four independent distal (cathode) electrodes, with a surface area of 5×5 cm^2 each, were placed distally (cathodes) in close proximity to each other, forming a 2×2 matrix just below the distal motor point of the RF muscle (see Fig. 2a). Positions of the electrodes were confirmed or modified based on visual observations and gentle touch of the muscle contraction resulting from a short train of pulses sent to each electrode separately. In order to minimize fatigue, the pulses used were slightly above the motor threshold. Pictures of the final electrode placement were taken to ensure accurate replication across sessions. The electrode configuration for Experiment 1 is shown in Fig. 2a.

In Experiment 2, the proximal (anode) electrode (5×9 cm^2) was placed on the motor point of the VL muscle. Four independent distal (cathode) electrodes, with a surface area of 2.5×4.5 cm^2 each, were placed around the distal motor point of the VL muscle. Similar to Experiment 1, the position of each electrode was tested separately and adjusted until the appropriate positioning was achieved. Pictures of the final configurations were taken to ensure consistent electrode positioning between sessions. The electrode configuration for Experiment 2 is shown in Fig. 2b.

In both experiments, the described electrode configuration was used for SDSS as well as SES. Unlike previous studies that have explored the topic of SDSS, in the SES mode, all four distal electrodes were connected to the cathode of the same stimulation channel effectively forming a single large electrode (indicated in Fig. 2 using electrode numeration 1,1,1,1). This was done to ensure that the electrode positioning and the area covered by the electrodes remained identical during SES and SDSS. Conversely, in the SDSS mode, each distal electrode was connected to a different output channel of the AFU device, thereby producing the SDSS effect (indicated in Fig. 2 using electrode numeration 1,2,3,4).

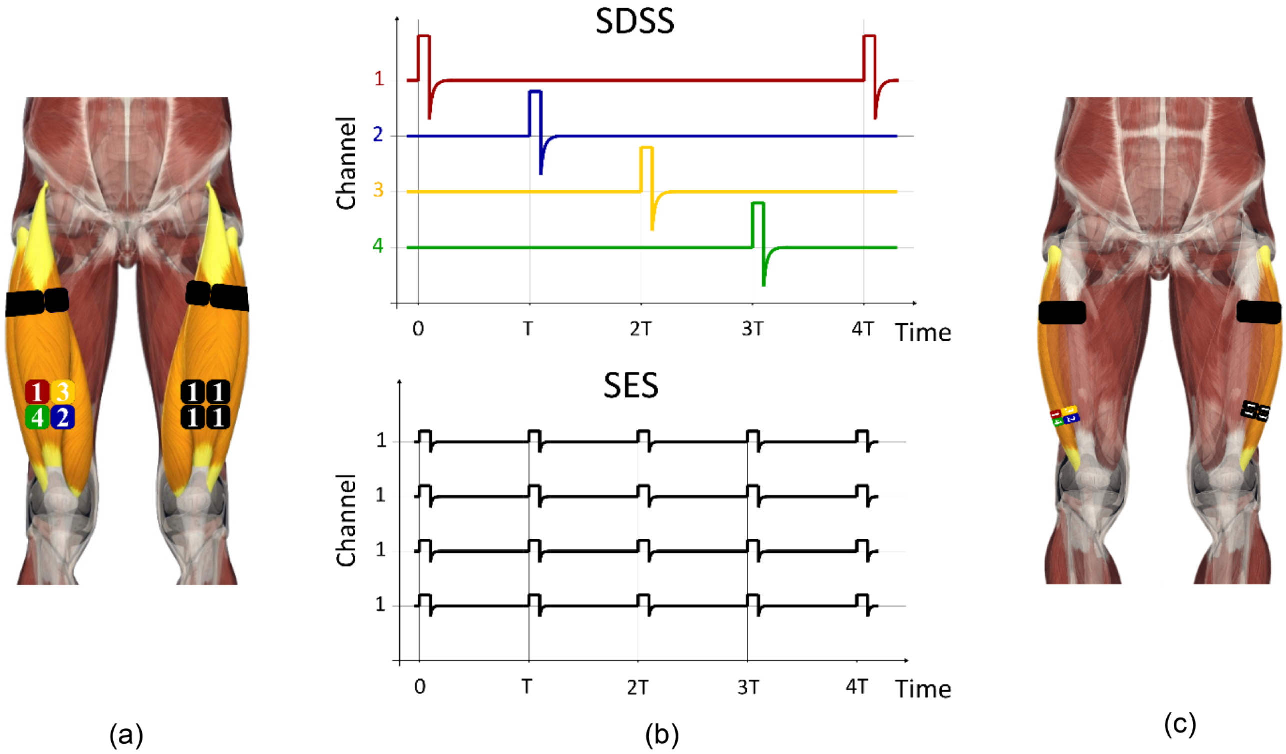

Fig. 2 Schematic representations of electrode configurations for **a**) Experiment 1 and **c**) Experiment 2. The middle panel **b**) illustrates typical electrical pulse trains for the SDSS and SES protocols, where the y-axis represents electrode channels, and the x-axis represents time (arbitrary units). The orange regions in the schematics indicate the stimulated muscle groups. The colors (red, blue, yellow, and green) in the SDSS plots serve as a reminder that different electrodes (regions around the motor point) are activated at different times. The total applied stimulation intensity using both configurations (SDSS and SES) was equal

Experimental protocol

All experiments were divided into two types of experimental sessions, one with high-intensity stimulation and the other with moderate-intensity stimulation, the sequence of which was chosen at random for each subject tested. To eliminate the possibility of residual fatigue from previous experimental sessions, a minimum of 48 h in between them was imposed. For each subject, before the first experimental session, SDSS was randomly assigned to one leg while SES was applied to the other leg. The chosen leg-stimulation arrangement was kept throughout all experimental sessions (Fig. 3).

During the experiments, the pulse width was set to 400 μs and the stimulation frequency was 48 Hz for SES and 12 Hz per channel for SDSS (composite frequency of 12 Hz x 4 = 48 Hz). Stimulation intensity was limited to 130 mA for Experiment 1 and 100 mA for Experiment 2. Stimulation parameters were based on the relevant literature [25, 26, 37, 38, 44] and slightly adapted according to the maximal stimulation parameters used by subjects with previous FES experience. Pre-study tests were performed to ensure fused contractions in the quadriceps muscle group and vastus lateralis muscle.

Each experimental session began with determining the high and moderate stimulation intensities for the quadriceps muscle group (Experiment 1) or vastus lateralis muscle (Experiment 2) on a randomly selected leg. High and moderate stimulation intensities were calculated based on the muscle's motor threshold intensity (lowest intensity at which the muscle produces a measurable force) and the force plateau intensity (lowest intensity at which the muscle produces maximal force). Using the SDSS configuration, stimulation pulses were sent with increasing intensity while the output force was measured. SDSS was selected to set the intensity for the force plateau because previous studies have shown that SDSS produces higher forces compared to SES at the same stimulation intensity [40, 45]. Every second, the stimulation intensity was programmatically increased in increments of 5 mA from 0 mA to 130 mA (Experiment 1) or from 0 mA to 100 mA (Experiment 2). If the force plateau was observed, the corresponding intensity was chosen as the high intensity. If the plateau was not reached, the maximum intensity of 130 mA (Experiment 1) or 100 mA (Experiment 2) was used. The moderate stimulation intensity was defined as the midpoint between the detected motor threshold intensity and the high stimulation intensity. After a 5-minute break, fatigue measurement was carried out by continuously applying

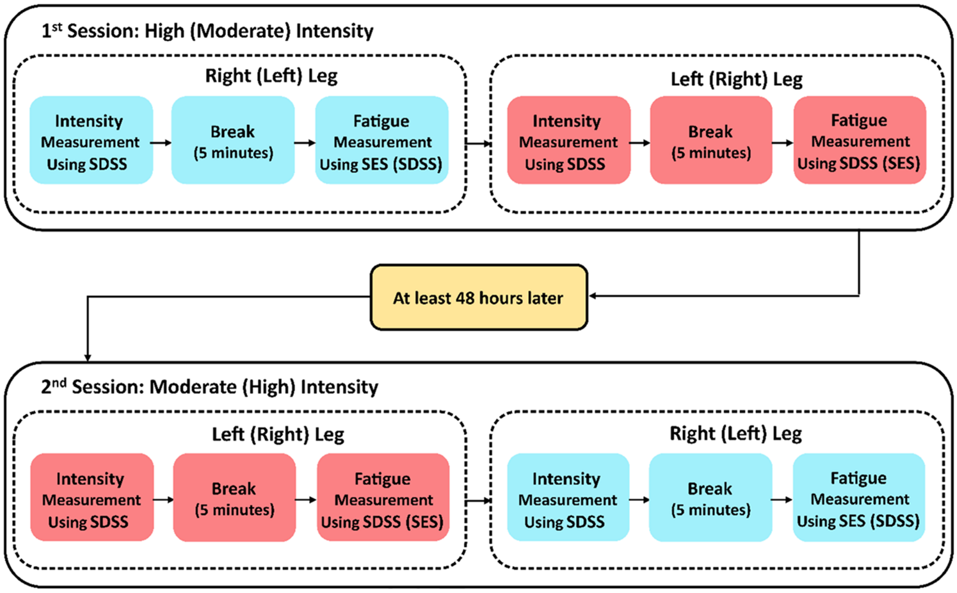

Fig. 3 Schematic of the randomized experimental protocol. Each experiment was divided into two sessions, one involving high-intensity stimulation and the other moderate-intensity stimulation, with the sequence randomized for each subject. A minimum of 48 h was maintained between sessions to prevent residual fatigue. Prior to the first session, SDSS was randomly assigned to one leg, while SES was assigned to the other, and this leg-stimulation arrangement was kept consistent across all sessions. Each session began with determining high and moderate stimulation intensities for the quadriceps (Experiment 1) or vastus lateralis (Experiment 2) muscles using SDSS configuration. Following a 5-minute break, fatigue measurements were conducted using the randomly assigned stimulation configuration (SDSS or SES). The process was then repeated on the opposite leg with the other electrode configuration. The second session mirrored the first, with the stimulation intensities for fatigue measurements being switched while maintaining the same leg-stimulation arrangement

the stimulation intensity (e.g., high intensity) chosen for the experimental session using the configuration (e.g., SES) assigned to the selected leg (e.g., right leg) until the measured force declined below 70% of the maximum produced force (MPF) value. Subsequently, using the SDSS configuration, high and moderate stimulation intensities were determined for the quadriceps muscle group (Experiment 1) or vastus lateralis muscle (Experiment 2) on the other leg (e.g., left leg). After a 5-minute break, fatigue measurement was carried out by applying the same stimulation intensity (e.g., high intensity) using the other electrode configuration (e.g., SDSS). After at least 48 h, the entire process was repeated for the second experimental session using the other stimulation intensity that was not used in the first session (e.g., moderate intensity) while maintaining the same leg-stimulation arrangement. The order in which the legs were tested was reversed between the two sessions to counterbalance any order effects (e.g., first session with high intensity: right leg-SES then left leg-SDSS; second session with moderate intensity: left leg-SDSS then right leg-SES). We applied the same intensity for SES and SDSS in the experiments to keep the amount of electrical charge at the exact same level for a fair comparison of the two stimulation methods.

Data analysis

The forces recorded during each fatigue measurement were analyzed using MS Excel (Microsoft Corporation, Redmond, WA, USA) and MATLAB (MathWorks, Natick, MA, USA). Maximum produced force (MPF) was defined as the maximum force value measured at the beginning of fatigue measurement tests using the assigned stimulation configuration, excluding any force peaks caused by spasticity. This approach aligns with the methods described in [25, 26]. Time to fatigue (TTF) was calculated as the time from stimulation onset to the moment the output force declined below 70% of the MPF. This 70% cutoff, as used in the works of Malešević et al. [25] and Popović and Malešević [26], balances sensitivity to fatigue onset while avoiding the risks of complete muscle exhaustion, particularly in SCI populations where variability in maximum force production is significant. The force-time integral (FTI) was calculated as the integral of the produced force over the period from stimulation onset to TTF. The average produced force (APF) was then calculated by normalizing FTI by TTF. APF provides an average value of force output over time, capturing both the magnitude of force and the duration of sustained contraction. This method ensures a comprehensive assessment of muscle performance under different stimulation conditions. The elicited outcome parameters are TTF, MPF, and APF. Any differences between using SDSS versus SES are calculated at the end of each experimental session using the following equations:

$$\%\mathrm{TTF}_{\mathrm{difference}} = 100 \times \frac{(\mathrm{TTF}_{\mathrm{SDSS}} - \mathrm{TTF}_{\mathrm{SES}})}{\mathrm{TTF}_{\mathrm{SES}}} \# (1)$$

$$\%\mathrm{MPF}_{\mathrm{difference}} = 100 \times \frac{(\mathrm{MPF}_{\mathrm{SDSS}} - \mathrm{MPF}_{\mathrm{SES}})}{\mathrm{MPF}_{\mathrm{SES}}} \# (2)$$

$$\%\mathrm{APF}_{\mathrm{difference}} = 100 \times \frac{(\mathrm{APF}_{\mathrm{SDSS}} - \mathrm{APF}_{\mathrm{SES}})}{\mathrm{APF}_{\mathrm{SES}}} \# (3)$$

As described in the previous section, the subjects participated in two types of experimental sessions, one with moderate intensity and the other with high intensity. The elicited outcome parameters were then classified into two groups based on the level of intensity for statistical analysis. To check the normality of the data and equivalence of the variance, the Shapiro-Wilk test and F-test were conducted, respectively. If the p-values for both tests were greater than 0.05, the two-tailed paired t-test was employed to identify any differences. Otherwise, the Wilcoxon signed-ranked test was applied. The p-values and d-values (Cohen's d) were used to indicate the significance and effect size of parametric tests. The p-values and r-values (calculated as the Z-score divided by the square root of sample size) were used for the same purpose in non-parametric tests. In this study, p-values lower than 0.05 indicated statistical significance.

Results

Experiment 1

Figure 4 provides an illustrative example of the extracted metrics and types of results obtained in this study, using data from a single participant during Experiment 1. This figure is intended to help the reader understand how the measurements were derived and interpreted.

Figure 4a and b depict the fatigue measurements of subject P3 for moderate electrical stimulation intensity (80 mA) and high electrical stimulation intensity (130 mA) conditions, respectively. During the first experimental session, the subject underwent high-intensity testing where the left quadriceps muscle group was stimulated using the SES configuration and the right quadriceps muscle group was stimulated with the SDSS configuration. In the subsequent experimental session, the moderate electrical stimulation intensity was tested with the same leg-stimulation arrangement (left leg-SES and right leg-SDSS). The horizontal black lines and shaded areas under the force-time curve represent TTF and FTI, respectively. Under moderate electrical stimulation intensity conditions, the TTF for SDSS and SES electrode configurations were 20.00 s and 11.47 s, respectively ($\%\mathrm{TTF}_{\mathrm{Difference}}$ = 74.37%), whereas, for high electrical stimulation intensity, the TTF for SDSS and SES electrode configurations were 13.18 s and 10.34 s, respectively ($\%\mathrm{TTF}_{\mathrm{Difference}}$ = 27.47%). For moderate electrical stimulation intensity, the FTI for SDSS and SES electrode configurations were 636.26 N·s and 111.01 N·s, respectively ($\%\mathrm{APF}_{\mathrm{Difference}}$ = 228.70%). For high electrical stimulation intensity, the FTI for SDSS and SES were 836.03 N·s and 408.85 N·s, respectively ($\%\mathrm{APF}_{\mathrm{Difference}}$ = 60.42%).

Figure 5a represents the $\%\mathrm{TTF}_{\mathrm{Difference}}$ values for all subjects under moderate and high electrical stimulation intensity conditions. The $\%\mathrm{TTF}_{\mathrm{Difference}}$ for moderate electrical stimulation intensity had a median of 118.83%, ranging from 51.90 to 262.44%, while the $\%\mathrm{TTF}_{\mathrm{Difference}}$ for high electrical stimulation intensity had a median of 40.28%, ranging from 9.84 to 110.28%. Statistical analysis reveals that the $\%\mathrm{TTF}_{\mathrm{Difference}}$ for moderate electrical stimulation intensity was significantly larger than that for the high electrical stimulation intensity tests (group

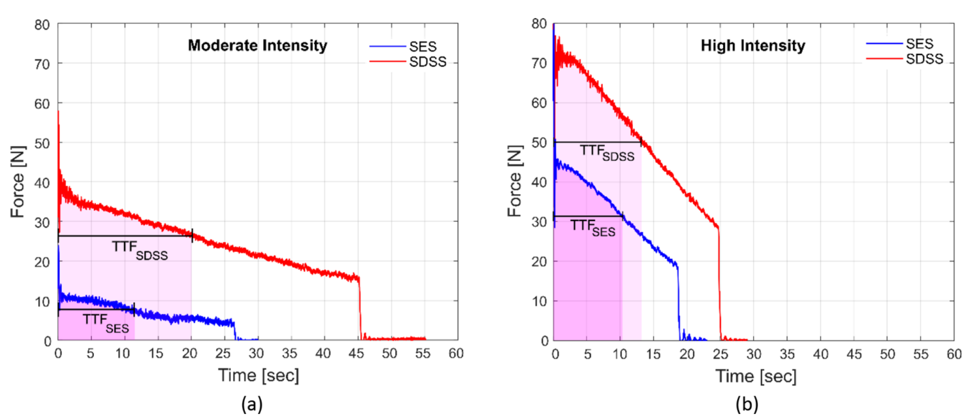

Fig. 4 Measurements of the force produced by the quadriceps muscle group in subject P3 during Experiment 1, under two conditions: **a**) moderate electrical stimulation intensity (80 mA) and **b**) high electrical stimulation intensity (130 mA). Forces produced by the quadriceps muscle group under SDSS and SES electrode configurations are represented by red and blue lines, respectively. Horizontal black lines indicate the time to fatigue (TTF), and the shaded areas under the force-time curves represent the force-time integral (FTI)

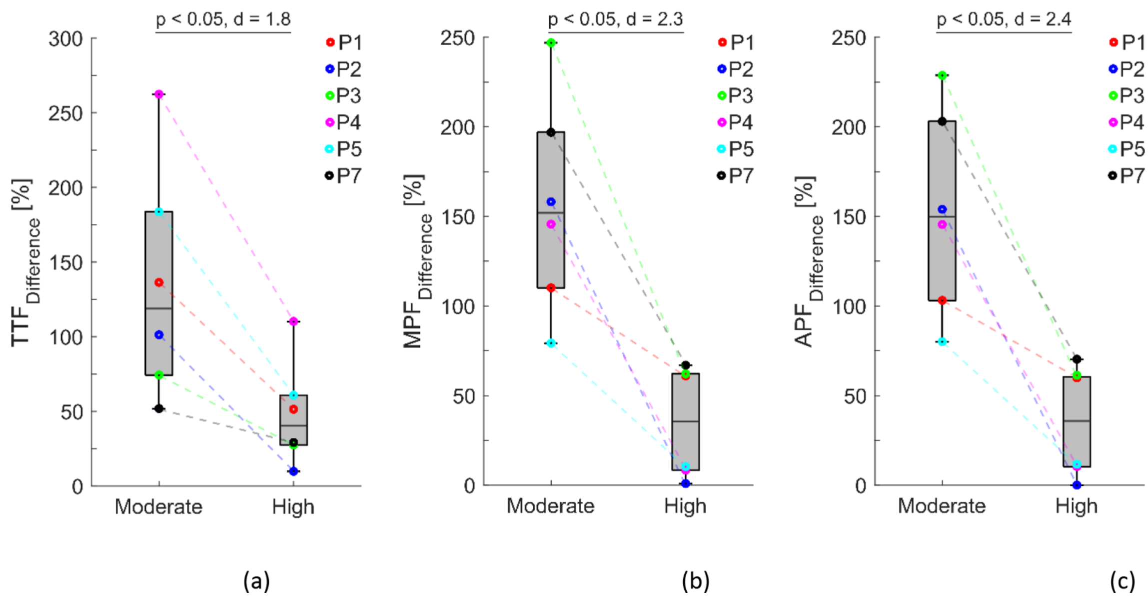

Fig. 5 Box-and-whisker plots summarizing the results from Experiment 1: **a**) $\%TTF_{Difference}$, **b**) $\%MPF_{Difference}$, and **c**) $\%APF_{Difference}$ values under moderate and high electrical stimulation intensity conditions. Values from each subject are presented in a different color

mean ± standard deviation, 135.01 ± 71.10% for moderate electrical stimulation intensity and 48.17 ± 32.37% for high electrical stimulation intensity), as determined by paired t-test ($p = 0.0065$, d = 1.8).

Figure 5b illustrates the $\%MPF_{Difference}$ values for all subjects under moderate and high electrical stimulation intensity conditions. The $\%MPF_{Difference}$ for moderate electrical stimulation intensity had a median of 151.92%, ranging from 79.28 to 246.92%, whereas the $\%MPF_{Difference}$ for high electrical stimulation intensity had a median of 35.68%, ranging from 0.88 to 66.98%. The $\%MPF_{Difference}$ for moderate electrical stimulation intensity was found to be significantly higher than that for high electrical stimulation intensity (156.19 ± 54.83% for moderate electrical stimulation intensity and 34.95 ± 28.60% for high electrical stimulation intensity), as determined by paired t-test ($p = 0.0023$, d = 2.3).

Figure 5c portrays the $\%APF_{Difference}$ values for all subjects under moderate and high electrical stimulation intensity conditions. The $\%APF_{Difference}$ for moderate electrical stimulation intensity had a median of 149.73%, ranging from 80.08 to 228.70%, while the $\%APF_{Difference}$ for high electrical stimulation intensity had a median of 35.81%, ranging from 0.08 to 70.33%. The $\%APF_{Difference}$ for moderate electrical stimulation intensity was significantly higher than that for high electrical stimulation intensity (152.40 ± 51.79% for moderate electrical stimulation intensity and 35.48 ± 28.53% for high electrical stimulation intensity), as determined by paired t-test ($p = 0.0022$, d = 2.4). The values of TTF, MPF, and APF for moderate and high electrical stimulation intensity with respect to the associated stimulation configuration (SDSS and SES) and the corresponding differences (i.e., $\%TTF_{Difference}$, $\%MPF_{Difference}$, and $\%APF_{Difference}$) are shown in Table 2.

Experiment 2

Figure 6 provides an illustrative example of the extracted metrics and types of results obtained in this study, using data from a single participant during Experiment 2.

Figure 6a and b depict the fatigue measurements of P5 under conditions of moderate electrical stimulation intensity (60 mA) and high electrical stimulation intensity (100 mA), respectively. In the initial experimental session, the subject underwent moderate-intensity testing where the left VL was stimulated with the SDSS configuration and the right VL was stimulated using the SES configuration. In the subsequent experimental session, the high electrical stimulation intensity was tested with the same leg-stimulation arrangement (right leg-SES and left leg-SDSS). The horizontal black lines and shaded areas under the force-time curve represent TTF and FTI, respectively. The TTF values for SDSS and SES electrode configurations under moderate electrical stimulation intensity were 17.90 s and 12.65 s, respectively, resulting in a $\%TTF_{Difference}$ of 41.50%. At high electrical stimulation intensity, the TTF values for SDSS and SES electrode configurations were 11.96 s and 11.22 s, respectively, resulting in $\%TTF_{Difference}$ of 6.60%.

Regarding FTI, at moderate electrical stimulation intensity, the values for SDSS and SES electrode configurations were 147.03 N·s and 68.01 N·s, respectively ($\%APF_{Difference}$ of 52.78%). At high electrical stimulation intensity, the FTI values for SDSS and

Table 2 The summary of the results from each subject from experiment 1 conducted on the quadriceps muscle group. Unshaded and shaded rows represent the results of the first and second sessions, respectively

Subjects	Intensity [mA]	SDSS			SES			Difference		
		TTF [s]	MPF [N]	APF [N]	TTF [s]	MPF [N]	APF [N]	TTF [%]	MPF [%]	APF [%]
P1	70	34.1	43.1	36.9	14.4	20.5	18.2	136	110	103
	130	17.3	201.6	178.0	11.4	125.2	111.3	51.4	60.9	59.9
P2	70	24.9	65.2	56.4	12.4	25.3	22.2	101	158	154
	130	17.3	220.9	191.4	15.7	219.1	191.3	9.8	0.88	0.08
P3	80	20.0	37.8	31.8	11.5	10.9	9.7	74.4	247	229
	130	13.2	72.1	63.4	10.3	44.5	39.5	27.5	62.1	60.4
P4	80	38.0	8.2	7.2	10.5	3.3	2.9	262	146	145
	130	19.6	23.8	20.9	9.3	21.9	18.9	110	8.4	10.4
P5	80	27.8	15.5	13.9	9.8	8.6	7.7	184	79.3	80
	130	14.6	42	37	9.1	38.1	33.1	61	10.4	11.7
P7	85	10.0	10.6	9.2	6.6	3.6	3.0	51.9	197	203
	130	7.9	12.5	11.1	6.1	7.5	6.6	29.1	67	70.3

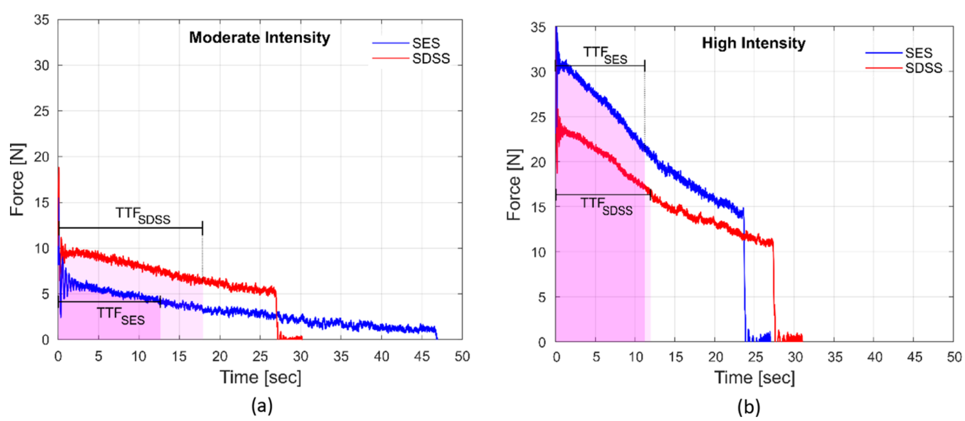

Fig. 6 Measurements of the force produced by the vastus lateralis muscle in subject P5 during Experiment 2, under two conditions: **a**) moderate electrical stimulation intensity (60 mA) and **b**) high electrical stimulation intensity (100 mA). Forces produced by the vastus lateralis under SDSS and SES electrode configurations are represented by red and blue lines, respectively. Horizontal black lines indicate the time to fatigue (TTF), and the shaded areas under the force-time curves represent the force-time integral (FTI)

SES electrode configurations were 246.96 N·s and 300.87 N·s, respectively (%$APF_{Difference}$ of -23.00%).

In Fig. 7a, the %$TTF_{Difference}$ values for all subjects are presented under conditions of moderate and high electrical stimulation intensity. The median %$TTF_{Difference}$ for moderate electrical stimulation intensity was 39.58%, ranging from − 62.17 to 45.59%. For high electrical stimulation intensity, the median %$TTF_{Difference}$ was − 0.80%, ranging from − 37.90 to 16.59%. Statistical analysis utilizing the Wilcoxon signed-ranked test revealed no significant difference between %$TTF_{Difference}$ for moderate and high electrical stimulation intensity ($p = 0.31$, $r = 0.45$).

Figure 7b depicts the %$MPF_{Difference}$ values for all subjects under conditions of moderate and high electrical stimulation intensity. The median %$MPF_{Difference}$ for moderate electrical stimulation intensity was 2.03%, ranging from − 51.83 to 98.98%. For high electrical stimulation intensity, the median %$MPF_{Difference}$ was 8.95%, ranging from − 26.72 to 69.64%. The statistical analysis indicated no significant difference between %$MPF_{Difference}$ for moderate and high electrical stimulation intensity (16.35 ± 54.15% for moderate electrical stimulation intensity and 15.97 ± 39.03% for high electrical stimulation intensity), as determined by paired t-test ($p = 0.99$, d = 0.01).

Figure 7c presents the %$APF_{Difference}$ values for all subjects under moderate and high electrical stimulation intensity conditions. The %$APF_{Difference}$ for moderate electrical stimulation intensity had a median of 0.79%, ranging from − 51.20 to 72.03%, while %$APF_{Difference}$ for high electrical stimulation intensity had a median of 6.00%, ranging from − 26.21 to 60.65%. Statistical analysis using a paired t-test showed no significant difference between %$APF_{Difference}$ for moderate and high electrical stimulation intensity (10.85 ± 45.63% for moderate electrical stimulation intensity and 13.61 ± 36.23% for high electrical stimulation intNensity) ($p = 0.91$, d = 0.05). The values of TTF, MPF, and APF for moderate and high electrical stimulation intensity with respect to the associated

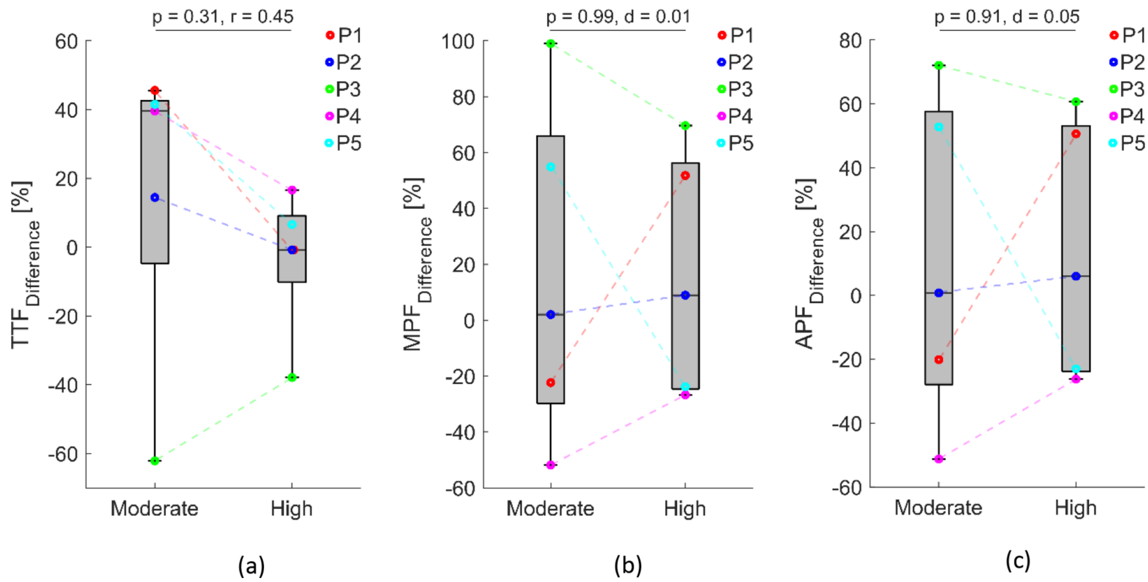

Fig. 7 Box-and-whisker plots summarizing the results from Experiment 2: **a**) $\%TTF_{Difference}$, **b**) $\%MPF_{Difference}$, and **c**) $\%APF_{Difference}$ values under moderate and high electrical stimulation intensity conditions. Values from each subject are presented in a different color

Table 3 The summary of the results from each subject from experiment 2 conducted on the vastus lateralis muscle. Unshaded and shaded rows represent the results of the first and second sessions, respectively

Subjects	Intensity [mA]	SDSS			SES			Difference		
		TTF [s]	MPF [N]	APF [N]	TTF [s]	MPF [N]	APF [N]	TTF [%]	MPF [%]	APF [%]
P1	60	12.87	29.7	27.4	8.84	38.2	34.3	45.6	-22	-20
	100	9.36	113.2	100.0	9.45	74.6	66.4	-0.9	51.8	50.6
P2	60	15.3	37.7	32.4	13.4	37.0	32.1	14.4	2.0	0.8
	100	13.7	85.6	77.6	13.8	78.6	73.2	-0.8	8.9	6.0
P3	65	2.8	23.4	17.3	7.5	11.8	10.0	-62	99.0	72.0
	100	5.2	50.3	42.0	8.4	29.7	26.1	-38	69.6	60.6
P4	70	13.9	1.8	1.67	10.0	3.8	3.4	39.6	-52	-51
	100	10.5	21.7	19.5	9.0	29.6	26.4	16.6	-27	-26
P5	60	17.9	9.4	8.2	12.6	6.0	5.4	41.5	54.9	52.8
	100	12.0	23.3	20.6	11.2	30.6	26.9	6.6	-24	-23

stimulation configuration (SDSS and SES) and the corresponding differences (i.e., $\%TTF_{Difference}$, $\%MPF_{Difference}$, and $\%APF_{Difference}$) are shown in Table 3.

Discussion

In the present study, we assessed the efficacy of spatially distributed sequential stimulation (SDSS) in comparison to the traditional FES using a single electrode stimulation (SES) with respect to the time to fatigue (TTF), maximum produced force (MPF), and average produced force until fatigue (APF) at moderate and high electrical stimulation intensities. The objective of each experimental session was to evaluate the efficacy of SDSS at the chosen stimulation intensity by comparing it to SES applied to the contralateral leg.

It is important to note that the described protocol was not designed to directly measure the fatigue-reducing capabilities of SDSS compared to SES. Instead, the purpose was to utilize SES as a reference to ensure a fair comparison of the effectiveness of SDSS across multiple experimental sessions. To further ensure the fair comparison of SDSS and SES ratios, we used the same amount of electrical charge to generate SES and SDSS. This resulted in smaller output forces for SES, which is in accordance with the conclusions of previous studies [40, 45]. We selected this strategy because the purpose of the study was to directly investigate the effect of the stimulation intensity on the performance of SDSS.

A common challenge encountered when conducting measurements over multiple days is the variation in the subject's strength, which can be influenced by various physiological and environmental factors. In this study, we applied SES to the same leg, considered as the reference, across both sessions to compensate for discrepancies in the subject's strength. Our rationale for this approach is grounded in the fact that all participants were individuals with motor-complete SCI, resulting in bilateral paralysis of the lower limbs. Unlike individuals with unilateral impairments (e.g., hemiplegic patients), both legs of SCI patients experience comparable levels of disuse and neuromuscular inactivity. Therefore, we assume that the physiological state of both legs is similarly influenced by day-to-day variations. It is important to clarify that we did not assume the physiological conditions of both legs to be identical, but rather that day-to-day variations induced by environmental factors would affect both legs in a comparable manner. Although this approach simplifies the experimental design and reduces participant burden, it introduces a potential limitation. Profiling both legs independently could provide a more comprehensive assessment of day-to-day and inter-limb variability and further strengthen the conclusions, particularly given the small cohort size of the study. However, due to time constraints and to minimize participant fatigue, this approach was not implemented in the current study.

Our research focused on the quadriceps muscle group and the vastus lateralis (VL) muscle of subjects with SCI. Our findings elucidated that the superior performance of SDSS over SES is considerably compromised for quadriceps muscle group under high-intensity modality. On average, the percentage increase in TTF observed during moderate-intensity stimulation was 2.80-fold greater than the corresponding value observed during high-intensity stimulation. Furthermore, in terms of the percentage increase in MPF and APF, the moderate-intensity modality exhibited a 4.47-fold and 4.3-fold greater increase, respectively, compared to the high-intensity modality. The superior performance of SDSS over SES is generally attributed to the asynchronous recruitment of motor units at lower frequencies. Additionally, applying the same amount of charge to a smaller area creates a higher charge density allowing for the resulting electrical fields to reach deeper regions. Asynchronous activation of adjacent motor units at a lower stimulation frequency (12 Hz instead of 48 Hz in this study) allows for longer rest periods for the individual motor units resulting in greater fatigue resistance of SDSS compared to SES. However, higher stimulation intensities generate stronger electrical fields beneath the electrodes, resulting in excessive recruitment of motor units in the overlapping areas. Consequently, this situation resembles the configuration of SES, leading to heightened fatigue of these motor units. To the best of our knowledge, while this phenomenon has been previously observed [38], it has never been investigated directly. Schmoll et al. conducted a study involving SCI individuals performing knee extension tasks and found no significant difference in terms of fatigue resistance between four different distributed stimulation configurations and single electrode stimulation configuration. They postulated that a certain amount of spillover could potentially account for these findings [38]. Agotici et al. found that the overlap of activated muscle fibers parallel to the electrodes increased with stimulation intensity using a computational approach. However, this case differed from the present experimental studies, as the simulation was performed on a smaller muscle, and the stimulation intensity was limited to 60 mA [40]. In a study conducted by Sayenko et al., a similar SDSS configuration to the current work was employed and compared to SES in 2-minute fatiguing isometric tests. They discovered that the mean peak torque during the initial 5 stimulations was not significantly different between SDSS and SES in both able-bodied and SCI individuals [44]. This observation could be attributed to the relatively high stimulation intensities utilized in both protocols, which diminishes the alternating stimulation effect of SDSS.

The primary objective of Experiment 2 was to examine the impact of the high intensity on the efficacy of SDSS compared to SES in individual muscles where the number of accessible motor points is limited, thereby potentially compromising the efficacy of the alternating recruitment strategy. In contrast to the findings from Experiment 1, our results obtained from the vastus lateralis muscle did not exhibit a significant difference between moderate and high-intensity modalities in terms of $\%TTF_{Difference}$, $\%MPF_{Difference}$, and $\%APF_{Difference}$. One possible explanation for this outcome could be attributed to the relatively high value of moderate intensity compared to the intensity values used in the study with similar electrode configuration [29] in which the spillover effect most likely did not occur [38]. However, given the relatively low output force of the vastus lateralis muscle and the presence of muscle weakness in individuals with SCI, such intensity selections were inevitable to ensure meaningful stimulation responses. Furthermore, in certain subjects, instances of co-contraction of the rectus femoris muscle were observed. This phenomenon may be attributed to two factors: the relatively high charge per pulse applied in relation to the size of the muscle and/or the oversized cathode electrode. The selection of electrode size for the vastus lateralis tests requires careful consideration due to the potential for inadvertently stimulating the motor fibers responsible for knee flexion, consequently inducing antagonistic stimulation of the thigh muscles. This delicate and sensitive nature of electrode sizing necessitates precise electrode placement and control to specifically target the intended muscle group and minimize any unintended activation of opposing muscle actions. Conducting further investigations with a larger sample size and repeating the tests using smaller electrodes on a muscle where co-activation of antagonistic muscles is less likely (e.g., tibialis anterior) could potentially elucidate the underlying cause for this observed outcome.

One limitation of the present study can be found in the randomization method used to ensure a balanced study design. In particular, Experiment 1 consisted of 6 subjects, 4 of whom were assigned high-intensity stimulation, while only 2 participants were assigned moderate-intensity stimulation in Session 1 (Table 2). Having an equal number of subjects using moderate and high electrical stimulation intensities in Session 1 would be preferable as it would introduce less bias to the results.

In contrast to previous studies [24–27, 33–36], the electrodes employed in both SDSS and SES tests were kept identical in size and shape (4 cathodic electrodes) to prevent inadvertent errors in electrode placement. To provide further clarification, previous investigations utilized a single electrode for SES, while employing four smaller electrodes to cover the same area as SES in SDSS. However, the curved edges of the small electrodes used in SDSS result in an unequal overall coverage area compared to the size of the single large electrode used in SES. Additionally, when utilizing a single large electrode, the heterogeneous nature of skin resistance can facilitate the preferential passage of electrical currents through more conductive regions, potentially influencing the outcomes in terms of recruited motor points and subsequently affecting fatigue and force generation. The utilization of four smaller electrodes ensures a uniform distribution of electrical charge among the electrode set. Further investigation is warranted to explore the potential disparities in fatigue and force outcomes between the utilization of four smaller electrodes and a single large electrode during SES. Our future research aims to address this knowledge gap and provide a comprehensive understanding of the implications associated with electrode configuration on fatigue and force generation.

The findings of this study suggest that the benefits of SDSS are more pronounced at moderate stimulation intensities but diminish at higher intensities due to overlapping activation of motor units. In the context of FES-based exercises, high stimulation intensities are typically required for sustained force output over extended durations. While this study focused on continuous isometric stimulation, which has a distinct fatigue profile, we believe that the stimulation spillover observed at high intensities would still compromise the benefits of SDSS when applied to FES-based exercises which typically have different fatigue profiles. During such activities, the muscles and motor points are constantly moving relative to the electrodes which may reduce or negate the spillover effect observed in the present study. Further investigation is needed to confirm the extent to which the spillover effect impacts performance in those scenarios.

The reduced efficacy of SDSS at high intensities may limit its application in tasks requiring sustained force output over long durations. The relationship between power output and task duration must therefore be carefully considered. If the goal is to produce more power output, higher intensities can be employed with the understanding that fatigue will occur more rapidly. Conversely, if the goal is to perform a task for a longer duration, the tradeoff between power output and fatigue resistance should guide the adjustment of stimulation intensity to a moderate level. Future research should explore how SDSS performs under different stimulation paradigms, particularly those involving high-intensity, long-duration tasks such as FES cycling, to better understand its practical applications.

Conclusion

The study demonstrated that stimulation intensity significantly influences the efficacy of SDSS compared to SES. The superior performance of SDSS over SES, characterized by greater fatigue resistance and force output, is notably diminished when high electrical stimulation intensity is delivered through electrodes placed in close proximity, supporting the role of overlapping motor unit activation. These findings should be taken into account when determining the objectives of functional tasks and contribute to the growing body of knowledge on distributed stimulation approaches and their potential applications in neuromuscular rehabilitation.

Author contributions

All authors contributed to the conception and design of the study. EJ and PK acquired the data and performed the data analysis, interpreted the results and drafted the manuscript. All authors edited and revised the manuscript and approved the final version.

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Data availability

The data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The project was conducted in accordance with the Declaration of Helsinki, approved by the local Institutional Review Board, and registered at ClinicalTrials.gov (NCT06421753). In addition, written informed consent was obtained from all subjects. The participants were assured that their information would only be used for analysis in this study. All methods were carried out under relevant guidelines and regulations.

Consent for publication
Not applicable as all participants have been de-identified.

Competing interests
The authors declare no competing interests.

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