1   **Non-invasive spinal electro-magnetic stimulation (SEMS): a tool for evaluation and**

2   **modulation of lower limb spinal-muscular transmission in healthy adults.**

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18   **Short title: Spinal magnetic stimulation for neurophysiological evaluation and plasticity at**

19   **leg neuro-muscular circuitry**

Abstract

Objective. Our earlier electrophysiological recordings using animal models revealed diminished transmission through spared fibers to motoneurons and leg muscles after incomplete spinal cord injury (SCI). Administration of spinal electro-magnetic stimulation (SEMS) at specific parameters induced transient improvement of transmission at neuro-muscular circuitry in SCI animals. In the current human study, we sought translate this knowledge to establish optimal parameters of SEMS for (i) neurophysiological evaluation via Compound Motor Action Potential (CMAP); and (ii) modulation at neuro-muscular circuitry via H-reflex and M-wave response in 12 healthy adults.

Methods. SEMS application was with a coil positioned over T12-S1 spinal levels. SEMS-evoked CMAP-responses were wirelessly measured simultaneously from biceps femoris (BF), semitendinosus (ST), vastus lateralis (VL), soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles. We also examined effects of SEMS trains on H-reflex and M-wave responses. H-reflexes and M-waves were measured simultaneously from SOL, MG and LG muscles and evoked by peripheral electrical stimulation of tibial nerves before and after each SEMS session.

Results. Spinal levels for SEMS application to evoke CMAP-responses in corresponding muscles and amplitude/latency of these responses have been established. SEMS applied over L4-S1 spinal levels at 0.2 Hz rate for 30 min induced facilitation of H-reflexes and M-responses. Facilitation lasted for at least 1 hour after stopping SEMS and was associated with a decrease in threshold intensity and leftward shift of recruitment curve for H-reflex and M-wave. SEMS did not alter TMS-evoked responses in hand muscles.

41 **Conclusion.** SEMS is a novel, non-invasive approach for sustained neuromodulation of H-reflex

42 and M-wave responses in triceps surae muscle group. The parameters of SEMS application

43 established in this study for evaluation and neuromodulation of neural pathways innervating leg

44 muscles in healthy individuals may be used as a reference for neurophysiological evaluation and

45 long-lasting plasticity of the lower limb spino-neuromuscular circuitry in individuals with SCI.

46

47

Introduction

Our recent intracellular recordings from individual axons [1] and motoneurons [2] revealed declined excitability in spared axons, impaired propagation of action potentials though these axons and thus diminished transmission to motoneurons and then to hindlimb muscles following chronic SCI in adult rats. Administration of spinal electro-magnetic stimulation (SEMS) trains induced strengthening of transmission at spino-neuromuscular circuitry in chronic SCI rats [3, 4]. The current study was designed to establish parameters of SEMS for neurophysiological evaluation and plasticity at neuro-muscular circuitry innervating various leg muscles in humans. In this initial phase, we have examined effects of SEMS in healthy adult individuals to obtain reference measures for further and on-going studies in SCI individuals.

In human physiology the electromyography (EMG), the soleus Hoffmann (H)-reflex and muscle (M) response have been traditionally used for monitoring the extent of muscle activity and neurophysiological diagnostics [5-7]. Another important neurophysiological diagnostic parameter is nerve-to-muscle conduction, which has been previously tested by either percutaneous [8], or direct needle stimulation [9] of spinal nerve roots. The remarkable ability of the electro-magnetic coil to deliver stimuli noninvasively and painlessly through skin and tissue to the deeply located peripheral nerves, has prompted several investigators to use this approach for physiological testing of the nerve-to-muscle circuitry [10-12]. This same coil has more recently been applied at thoracic spinal levels (SEMS) to evoke compound muscle action potentials (CMAPs) in leg muscles [13-15] and conditioning of these responses with peripheral electrical stimulations [14] has been examined.

69   Electro-magnetic stimulation is based on the principle of electromagnetic induction of an

70   electric field through intact tissue to underlying structures systems [16-17]. Transcranial electro-

71   magnetic stimulation (TMS), since its initial introduction about three decades ago [18], has been

72   widely used for diagnostic applications and repetitive TMS was found to alter excitability at

73   cortico-motor circuitry [17, 19-20]. Responses evoked by TMS, however, are reliably recorded

74   from arm but not leg muscles, particularly in human participants with neurological impairments,

75   including spinal cord injury [21]. Consistent with these reported results of human studies, our

76   recent experiments using animal models revealed that TMS-evoked responses could be reliably

77   recorded from the hind limb muscles only in the naïve animals, but not in adult rats with SCI [22].

78   However, responses evoked by electro-magnetic stimulation at spinal thoracic and lumbar levels

79   could be reliably recorded from hind limb muscles in rats with chronic SCI [1]. In fact, EMG

80   recordings from the hind limb muscles, conducted in parallel with intracellular

81   electrophysiological recordings from individual motoneurons and axons in lumbar spinal

82   segments, revealed that spinal electromagnetic stimulation is an excellent non-invasive approach

83   for evaluation and modulation of transmission in spinal and spino-neuromuscular circuitry in adult

84   rats [1, 4, 22].

85   Various types of functional outcomes induced by repetitive SEMS applied at different

86   spinal levels, using different parameters and frequencies, have been reported as well. High-

87   frequency stimulation using the EMS coil positioned over T11-T12 vertebrae induced involuntary

88   bilateral locomotor-like movements in healthy individuals [13]. Lumbar repetitive magnetic

89   stimulation was reported to reduce spastic tone of the lower limbs in spinal cord injury and multiple

90   sclerosis patients [23, 24]. SEMS has been used as a treatment for urinary frequency and urge

91   incontinence [25] as well as to suppress detrusor contraction [26]. SEMS has been used effectively

92   to stimulate the spinal nerves in spinal cord injury individuals, resulting in some improvement of

93   several vital functions [15, 27-29]. Research has also demonstrated greater effects of magnetic

94   stimulation versus electrical stimulation in inhibition of detrusor hyperactivity [30]. Another study

95   reported that repetitive magnetic stimulation at spinal levels, in combination with motor training,

96   induced an acute and persistent decrease of low back pain and was more beneficial that motor

97   training alone [31].

98   However, the CMAP responses recorded from various leg muscles and evoked by SEMS

99   applied at different spinal levels have not been systematically examined and compared. Effects of

100   SEMS on H-reflex and M-responses of the leg muscles have been understudied.

101   The first objective of the current study was to examine the ability of single-pulse SEMS to

102   deliver electrical excitation to a variety of leg muscles in healthy adults. Using a wireless EMG

103   system, the SEMS-evoked responses were recorded simultaneously from biceps femoris (BF),

104   semitendinosus (ST), vastus lateralis (VL), and soleus (SOL), medial gastrocnemius (MG) and

105   lateral gastrocnemius (LG) muscles in both legs. The second objective was to examine the ability

106   of SEMS applied for 30 mins. to affect spino-neuromuscular plasticity: we compared M-wave and

107   H-reflex responses that were recorded simultaneously from triceps surae muscles (SOL, MG and

108   LG), before, immediately after the termination of SEMS, and 1 hour afterwards. We have also

109   examined whether SEMS applied repetitively would alter TMS responses recorded from hand

110   muscles. Some of these findings have been reported in abstract form [32].

111

112

Material and methods

Participants

Twelve healthy adult volunteers with no known history of neurological disorders, four male veterans (mean age 57.7 ± 7.5 years) participated in the study at the Northport VA Medical Center and 4 females (mean age 33.75 ± 9.67 years) and 4 males (mean age 26 ± 2.16 yeas) participated in the study at the Stony Brook University. All participants were screened for inclusion/exclusion criteria, gave written informed consent to participate in this study and completed up to 3 sessions. Participants were excluded from the study if they had any metal implants, any implanted electrical devices, any medications that could raise seizure threshold, cardiac conditions, history of syncope or concussion with loss of consciousness, or ringing in the ears. All females were provided a pregnancy screen where negative results allowed participation. All participants were advised not to exercise on the day of the study. This study was approved by the local Institutional Review Board (IRB) at Stony Brook University and Northport VA Medical Center and was conducted in accordance with the Declaration of Helsinki.

Overview of Study Design

Blood pressure and heart rate were measured before and after administration of each SEMS session. First, TMS induced responses in the hand muscles were measured followed by baseline measurements of M-wave and H- reflexes recruitment curves of the leg muscles using peripheral nerve stimulation of the tibial nerve (see below). After the baseline (i.e. prior SEMS application) measurements were taken, a 30 minute session of SEMS applied in repetitive trains, was

135   administered (see below). After the SEMS administration, M-wave and H-reflex recruitment

136   curves and TMS induced responses were again collected for comparison with the pre-SEMS

137   baseline measurements. To evaluate the possible long lasting effects of SEMS, the same

138   measurements were retaken 1 hour post-SEMS administration in a subset of participants (n=4). At

139   the Northport VAMC the study design did not include TMS administration. This sequence was

140   repeated once a week for 3 weeks to total 3 visits.

141

142   **Electrophysiological recordings**

143   Electrophysiological recordings were performed using Delsys Trigno wireless EMG

144   system (Delsys Inc. Natick, MA). After standard skin preparation, wireless Trigno surface sensors

145   were placed over the muscle belly near the motor point and aligned with the muscle striations.

146   Lower limb recording electrodes were placed at the beginning of each session and were secured in

147   place for the entire session, without removal. During the first session, the optimal position for

148   placement of the recording electrodes was determined for each participant and was used for the

149   follow-up sessions.   For repeatability of EMG electrode placement for subsequent days,

150   measurements were taken from the knee crease with a tape measure to the EMG electrode location

151   and supported further with photographs of the electrode placement. For peripheral nerve

152   stimulation followed by SEMS, recording electrodes were placed on the soleus (SOL), medial

153   gastrocnemius (MG), lateral gastrocnemius (LG), vastus lateralis (VL), semitendinosis (ST) and

154   biceps femoris (BF) muscles bilaterally. We recorded from all these leg muscles during SEMS but

155   only triceps surae (SOL, MG and LG) for evaluation of H- and M-responses. For the TMS testing,

156   recording electrodes were placed on first dorsal interosseous (FDI) and abductor digiti minimi

157   (ADM) muscles. The EMG signals were recorded via Digidata 1440 digitizer (Molecular Devices,

158   AXON CNS) at 5 kHz sampling rate using Clampex 10.6 software and analyzed off-line using

159   Clampfit 10.6 software. The EMG responses were averaged and peak to peak amplitude from the

160   mean response was calculated for analysis.

161

Transcranial magnetic stimulation

163   Participants were comfortably seated in a chair with target arms well supported and

164   completely relaxed. MagStim 200^2 magnetic stimulator (Jali Medical, Inc. Woburn, MA) with

165    $D70^2$ magnetic coil attached to a coil stand was used for stimulation (see Fig 1). TMS was delivered

166   to the optimal scalp position to elicit maximal responses in contralateral first dorsal interosseous

167   (FDI) and abductor digiti minimi (ADM) muscles. After determining resting threshold intensity

168   required to evoke responses of at least 50 µV in 3 out of 5 stimulations the stimulator output was

169   set 20% above threshold intensity and at least 10 responses were recorded for analysis. TMS

170   evoked responses were recorded before SEMS and after for each participant at Stony Brook

171   University (n=8).

172   **Figure 1.** Photos are of sample participant during the experiments demonstrating the position of

173   recording electrodes. (A) receiving SEMS administration over spinal levels and peripheral

174   electrical stimulation over tibial nerves of both legs. (B) receiving TMS administration over the

175   left motor cortex while recording from right hand muscles.

176

Peripheral nerve stimulation, H-reflex and M-wave recordings

178   For peripheral nerve stimulation participants were placed in the prone position (Fig 1). All

179   participants were instructed to refrain from voluntary contraction of the leg muscles and there was

180   no background contraction during recordings. The M-wave and H-reflex responses were measured

181   before and after SEMS application in all 12 participants. Peripheral electrical stimulation was

182   delivered using Digitimer DS7A (Digitimer North America, LLC) constant current stimulator with

183   bipolar gold-plated bar electrode (Natus Neurology, Inc.) placed over tibial nerve at the popliteal

184   fossa. The optimal position for the stimulating electrode was based on the H-reflex and M-

185   responses elicited and the popliteal stimulating electrode was secured in place for the duration of

186   the session. The H-reflex and M-waves were recorded in all three triceps surae muscles using 1ms

187   duration square-wave pulse delivered with 0.2 Hz frequency. Beginning with a low intensity

188   stimulus that was then gradually increased until the threshold intensity for H-reflex was

189   determined. The stimulus intensity continued to be increased in small increments (1mA) until the

190   H maximum responses and later the M maximum responses were acquired.   These data allowed

191   for the plotting of the full stimulus/response recruitment curve. At each stimulus intensity, at least

192   6-10 responses were recorded. For analyses of the effects of SEMS on the recruitment curve the

193   responses recorded from the soleus muscle were used. Six of the triceps surae EMG responses

194   were averaged to measure the peak-to-peak amplitude for both the M-wave and H-reflex at each

195   intensity. The data were tabulated for the baseline pre- and post-SEMS and for both left and right

196   legs and used in the statistical analysis.

197

198   **Spinal electromagnetic stimulation (SEMS)**

199   Participants were placed in the prone position with comfortable support of a pillow under

200   the abdomen when preferred. SEMS was administered using MagStim 200^2 magnetic stimulator

201   (Jali Medical, Inc. Woburn, MA) with figure-of-8 $D70^2$ magnetic coil delivering monophasic

202   pulses at 0.2Hz frequency. The coil was always positioned with the handle towards the head to

203   standardize the direction of electro-magnetic stimulation. The optimal spinal level and stimulus

204   intensity were determined in their first session. To accomplish this, a total of 6-10 single pulses

205   were delivered to each spinal level from T12 to S1 and the stimulus intensity with 10% increments

206   ranged from 40% to 80% was applied until EMG recordings from the greatest number of muscles

207   were visible. After the optimal spinal level and SEMS intensity were determined, they were used

208   to deliver a 30 minutes of single pulse SEMS that was applied repetitively with 0.2 Hz frequency

209   for each of the three sessions. Spinal levels used for repetitive SEMS ranged from L4-L5 with 60-

210   80% of stimulus intensity. Six of all muscle consecutive responses have been collected and

211   averaged at each intensity and spinal level, then the peak-to-peak amplitude were measured and

212   tabulated for statistical analysis. The latency was measured from the beginning of stimulation to

213   the onset of the response.

214

Statistical Analysis

216   SigmaPlot 11.0 software (Systat Software, San Jose, CA) and SAS v9.3 (the SAS Institute,

217   Cary, NC) were used for statistical analyses. For each participant the maximum H-reflex and M-

218   response amplitudes before SEMS were normalized to 100% and the percent change for each

219   response was calculated after SEMS. Similarly, the threshold intensity for H-reflex before SEMS

220   was considered as 1 and used to normalize applied intensities before and after SEMS. To compare

221   the measured characteristics of H-reflex and M-wave for pre-SEMS vs post-SEMS, paired t-test

222   or Wilcoxon Signed Rank test were used and the results were considered statistically significant

223   for $p < 0.05$. Data are presented as means $\pm$ standard errors.

224   To analyze the recruitment curves of H-reflex and M-wave, the ascending part of the

225   stimulus/response recruitment curve for SOL muscle has been fitted using 3-parameter sigmoid

226   function (SigmaPlot 11.0) with following equation for each individual [33]. Parameters such as

227   the slope of the recruitment curve and stimulus intensity needed to obtain 50% of the maximum

228   responses (S50) were obtained by analyses of the computer-fitted recruitment curves.

229
$$H(s) = \frac{Hmax}{1 + \exp\left(\frac{S50 - S}{b}\right)}$$

230   Where S is the stimulus intensity, Hmax is maximum amplitude of H-reflex. S50 is stimulus

231   intensity needed to obtain 50% of Hmax, and b is the steepness of the curve. In addition, we

232   calculated the slope of the curve at S50 using following equation

233
$$Hslope = \frac{Hmax}{4b}$$

234   The M-wave recruitment curves were analyzed using the same equations. The amplitudes

235   were calculated as peak to peak amplitude and intensities were normalized to pre-SEMS threshold

236   intensity for H-reflex. i.e. for each participant/curve pre-SEMS threshold intensity for H-reflex

237   was considered as motor threshold (MT). Because of substantial difference in actual amplitude of

238   H- and M-responses among subjects the amplitudes were normalized to 100% of H-Max and M-

239   Max, respectively, for each subject. All parameters analyses were grouped from each subject first

240   and then averaged among subjects. Statistical analyses of the fitted curves were performed using

241   SAS ver. 9.3 (the SAS Institute, Cary, NC). Descriptive statistics (e.g. means and standard

242   deviations) of all above mentioned parameters were calculated for pre-, post- and pre-post SEMS

243   change. The average SOL H-reflex and M-wave recruitment curves were generated using average

244 curve parameters ± standard errors (e.g. Max, b, and S50). Three-way repeated measure ANOVA

245 models (rmANOVA) were used to determine the effects of SEMS (pre- vs post-SEMS). Since the

246 effects of SEMS was the primary study aim, SEMS (pre- vs post-SEMS) remained in the final

247 models regardless of its statistical significance. The p values were then calculated based on the F

248 tests of rmANOVA for all parameters. Due to the small sample size and the exploratory nature of

249 this study, the primary purpose of the analysis was to examine mean trends. The conclusions will

250 not be purely based on a significant p values [34]. Adjustment for p values depends on the number

251 of tests and will increase the type II error [35]. Therefore, the p values were not adjusted for

252 multiple tests [36] and any p values less than 0.05 were considered statistically significant. The

253 correlations between M-wave and H-reflex parameters were estimated and tested for pre-, post and

254 pre-post change of SEMS.

Results

255

SEMS-evoked CMAP responses

256

257   In this study we have examined the non-invasive electromagnetic stimulation (SEMS)

258 administered at spinal levels in healthy participants. SEMS was administered at different spinal

259 levels (T12-S1) and at different stimulus intensities (40 % - 80%). Fig 2 includes representative

260 responses recorded from biceps femoris (BF), vastus lateralis (VL), semitendinosus (ST), medial

261 gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (SOL) muscles from one participant.

262 Not surprising, the administration of SEMS at different spinal levels results in the activation of

263 muscles differently.

264

Figure 2. Spinal electromagnetic stimulation (SEMS) was able to evoke compound muscle action potential (CMAP) responses in several leg muscles in healthy participants. Representative responses recorded from vastus lateralis (VL), biceps femoris (BF), semitendinosus (ST), soleus (SOL), lateral gastrocnemius (LG) and medial gastrocnemius (MG) muscles are presented. Note different muscles exhibit different size and latencies in response to SEMS. Diagram illustrating the position of the SEMS stimulation coil and recording electrodes.

SEMS administration at T12-L1 levels evoked responses predominantly in BF and VL muscles (Fig 2). When SEMS was administered at low lumbar levels (L3-L5), it resulted in activation of all triceps surae muscles (MG, LG and SOL), as well as BF and ST muscles in some participants (Fig 2). Amplitude and latencies of these responses were different depending on the spinal level of stimulation and the recorded muscle. These results indicate that SEMS administration at specific spinal level results in activation of spinal different pathways to the muscles.

With the same stimulation intensity, the optimal spinal level to evoke CMAP responses in the triceps surae muscle group (MG, LG, SOL) was the L5-S1 spinal levels. The mean amplitude for the SOL muscle at 70% of the maximum coil intensity was 0.15 ± 0.02 mV whereas the MG and LG muscles showed 0.14 ± 0.3 mV and 0.10 ± 0.02 mV amplitude respectively (n=7). For the VL muscle, the optimal spinal level was T12-L1. The mean amplitude for the VL muscle at 70% of maximum coil intensity was 0.14 ± 0.07 mV. For the BF and ST muscles, the optimal spinal stimulation level was L1-L3. The mean amplitude for BF muscle at 70% of maximum coil intensity was 0.03 ± 0.01 mV and ST was 0.05 ± 0.01 mV respectively.

286   Latencies of CMAP responses were also different depending on the muscle. Latency for

287   the VL muscle responses evoked from L1 SEMS was 11.8 ± 1.1 ms, 13.5 ± 1.3 ms for BF muscle

288   and 12.8 ± 1.9 ms for the ST muscle. Latency of responses evoked by coil positioned over L5 for

289   triceps surae muscles showed slightly longer latencies, i.e. 15.3 ± 0.53 ms for MG; 14.7 ± 0.45 ms

290   for LG and 15.4 ± 0.54 ms for SOL. The CMAP responses from the same muscles exhibited

291   different latencies and different amplitudes when the SEMS coil was moved caudally. In the same

292   participant, the CMAP responses recorded from SOL muscle showed decreased latency and

293   increased amplitude as the SEMS coil was adjusted caudally. For example, in one participant the

294   latency at L1 was 21.2 ms but at the L5 level was 18.1 ms and the amplitude was 0.01mV at L1

295   and 0.21mV at L5 respectively.

296

297   Effects of single pulse SEMS with repetitive trains on M-wave and H-reflex

298   amplitudes

299   We have examined effect of SEMS administration for 30 min on the M-wave and H-reflex

300   responses. We measured the M-wave and H-reflex response in all triceps surae muscles before and

301   after administration of SEMS train (see methods). The H-reflex and M-wave responses elicited by

302   electrical stimulation of the tibial nerve at the popliteal fossa can be observed in Fig 3A. The

303   responses recorded simultaneously from the SOL, MG and LG muscles, varied among participants.

304   Fig 3B shows the M-wave and H-reflex responses recorded from the same participant immediately

305   after SEMS administration using same current intensity as in Fig 3A. Both H- and M-responses

306   exhibited facilitation in all three muscles after SEMS. Similar facilitation after SEMS

307   administration was observed in all 12 participants tested. Since the SOL muscle has been examined

308   extensively in the literature, the motor threshold (MT), or the minimum stimulus intensity that

309   produced a minimal measurable H-response (about 30 µV in at least 5 of 10 trials) in the SOL

310   muscle was used for further analyses. This effect of SEMS on H-reflex and M-response was

311   associated with changes in threshold intensities for both H- and M-responses (see below). There

312   was no change in the latencies of both H-and M responses after SEMS administration. Before

313   SEMS the mean latency for SOL muscle of M response was 8.15 ± 0.32ms and of H was 28.5 ±

314   0.42ms; after SEMS administration latency for M was 8.18 ± 0.32ms and for H was 28.8 ± 0.41ms

315   (p > 0.05; n=12).

316   **Figure 3. Modulation of H-reflex and M-wave by SEMS train in healthy participants.** After

317   repetitive SEMS (0.2 Hz for 30 min) amplitude of both H-reflex and M-wave responses show

318   marked facilitation in all three soleus (SOL), Medial Gastrocnemius (MG) and Lateral

319   Gastrocnemius (LG) muscles. (A) Representative traces of H-reflex and M-wave responses

320   recorded from SOL, MG and LG muscles before (A) and after SEMS train (B) from same

321   participant evoked with same current intensities stimulating tibial nerve (approx. 20% above motor

322   threshold). Diagram illustrating the position of the stimulation electrode for tibial nerve stimulation

323   and recording electrodes for H-reflex and M-wave.

324   In two participants, we examined whether changes in positioning of the figure of-eight

325   shaped electro-magnetic coil over human vertebrae would affect amplitude of SEMS-evoked

326   CMAP responses, or SEMS-induced modulation of M- and H-responses. Rotating the coil 180°,

327   with the handle positioned towards the legs instead of towards the head, while keeping its center

328   at same spinal level, did not induce changes in the amplitude of SEMS-evoked responses.

329   Additionally, it did not affect the ability of SEMS administration to modulate the M-wave and H-

330   reflex responses. These results are consistent with previous reports indicating that (i) current

331 flowing in a clockwise or anticlockwise directions did not induce a difference in the amplitude of

332 the responses in humans [14], (ii) rotation of the coil 180°, did not induce significant changes in

333 the effects of SEMS on synaptic transmission in animal models [1], (iii) neither it affected

334 movement of the surgical rods [37]. However, all data represented are from participants receiving

335 SEMS with the coil cable positioned cephalad.

336

Effects of single pulse SEMS administration with repetitive trains on Motor Threshold (MT) of M-wave and H-reflex

339   We have also systematically examined the effects of SEMS administration on the threshold

340 intensity required to evoke H-reflex and M-wave responses. Fig 4A demonstrates representative

341 traces of M-wave and H-reflex responses, recorded at different stimulus intensities in SOL muscle

342 from one participant before and after administration of SEMS. After SEMS administration (for 30

343 min at 0.2 Hz), the threshold intensity for both H- and M-responses was significantly lower. Full

344 intensity/response recruitment curve for the SOL muscle for the same participant is presented in

345 Fig 4B (participant 1). After SEMS administration, there was a leftward shift (to lower intensity)

346 for both responses. A similar shift of the recruitment curve and decrease of threshold intensity was

347 observed in all participants. Representative recruitment curves from randomly chosen two other

348 participants (participant 2 and participant 3), are presented in Fig 4B as well. Results revealed that

349 administration of SEMS for 30 minutes induced a substantial leftward shift of the recruitment

350 curve for both H- and M-responses; however, this shift was different among participants (Fig 4).

351 SEMS-induced changes of H-reflex and M-wave responses in SOL muscle were compared in each

352 participant first and then grouped among participants. The mean threshold intensity for H-reflex

353   was 12.4 ± 1.1 mA pre-SEMS vs 8.8 ± 0.7 mA post-SEMS, i.e. 73 ± 3% of pre-SEMS control (P

354   < 0.05, n = 7). The mean threshold intensity for M-wave was 17.4 ± 1.6 mA pre-SEMS vs 12.1 ±

355   1.2 mA post-SEMS, i.e. 70.84 ± 4.31% of pre-SEMS control (P < 0.05, n = 7).

356   **Figure 4. Effect of SEMS train on recruitment curve of H-reflex and M-wave responses.** (A)

357   Representative traces of H-reflex and M-wave responses recorded from SOL muscle in one

358   participant (participant 1) before and after SEMS. After SEMS there is a dramatic change in the

359   current required to evoke H-reflex. Representative traces of H-reflex and M-wave are presented

360   for each stimulation current intensity before and after SEMS. (B) Representative stimulus/response

361   recruitment curves for H-reflex and M-wave for the same (participant-1) and two other

362   (participants 2 and 3) participants before and after SEMS demonstrating a leftward shift of both

363   H- reflex and M-wave curves.

364

365   **Statistical analyses of the recruitment curves for H- and M-responses**

366   A summary of the results demonstrating changes in characteristics of M-wave and H-reflex

367   responses in SOL muscle following administration of SEMS train (30 min, 0.2 Hz) is presented in

368   Fig 5. In order to generalize results of the SEMS effects on the H- and M- recruitment curves in

369   all participants it was important to account for the differences in MT intensity and amplitude of H-

370   and M-responses among participants. Therefore, the stimuli intensities were normalized to the pre-

371   SEMS values of the MT; and amplitudes of H- and M-responses were normalized to 100% of

372   maximum responses pre-SEMS, respectively, for each participant. The rising phase of recruitment

373   curves for H-reflex and M-wave was fitted using sigmoid function and analyzed (see methods).

374 **Figure 5.** Computer-generated analyses to summarize results of the effects of SEMS trains on

375 recruitment curves for M-wave and H-reflex responses among all participants. (A) Plot

376 demonstrating H-reflex recruitment curves using three parameter sigmoid fits for each participant.

377 Solid lines demonstrate sigmoid fits before SEMS and doted lines demonstrate H-reflex sigmoid

378 fits after SEMS for each participant. (B, C) Averaged curves for H-reflex and M-wave before

379 (solid lines) and after SEMS administration (dotted lines) respectively. Shaded area represents

380 standard error.

381   Analyses of the parameters of fitted M-wave curve are summarized in Table 1. The slope

382 at 50% M-max was flatter after SEMS compared to before SEMS (157.51 ± 76.42 pre-SEMS vs

383 139.78 ± 53.95 post-SEMS, $p=0.03$, Mean $\pm$ SD). S50 parameter before SEMS was significantly

384 greater than S50 after SEMS (1.98 ± 0.44 pre-SEMS vs 1.81 ± 0.59 post-SEMS, $p=0.03$, Mean $\pm$

385 SD). However, M-max, b, and intensity range were not significantly different before compared to

386 after SEMS.

387

388   **Table 1. Descriptive Statistics of Parameters for M Wave, Pre and Post SEMS**

389

Parameter	Pre N=19 Mean±SD Median (range)	Post N=19 Mean±SD Median (range)	Change (Post-Pre SEMS) N=19 Mean±SD Median (range)	p-value[1]
M-max	101.8±10.0 100.09 (93.81, 140.82)	103.03±8.5 100.64 (94.17, 132.95)	1.3±4.6 0.73 (-7.87, 11.22)	0.45
b (Steepness)	0.2±0.2 0.2 (0.07, 0.8)	0.2±0.2 0.16 (0.12, 0.87)	0.02±0.04 0.01 (-0.06, 0.08)	0.10
Mslope (Slope at 50% M-max)	157.5±76.4 142.5 (44.21, 330.42)	139.8±54 159.03 (40.37, 207.57)	-17.7±55.5 -4.07 (-143.94, 59.91)	0.03
Intensity corresponding to M-max (x MT)	3.0±0.6 2.8 (2.42, 4.14)	2.8±0.78 2.6 (1.79, 4.43)	-0.2±0.3 -0.2 (-1.03, 0.40)	0. 05
Intensity range (Max - Min) (x MT)	2.0±0.6 1.8 (1.42, 3.14)	2.0±0.7 1.7 (1.16, 3.5)	-0.04±0.2 -0.05 (-0.45, 0.36)	0.60
S_{50} (Intensity corresponding to 50% M-max) (x MT)	2.0±0.4 1.9 (1.51, 3.36)	1.8±0.6 1.7 (0.94, 3.35)	-0.2±0.3 -0.1 (-0.75, 0.22)	0.03

390   [1]Testing the significance of pre-post change based on F-tests of rmANOVA.

391

392   Analyses of the parameters of fitted H-reflex curve are summarized in Table 2. The

393   stimulus intensity corresponding to H-max before SEMS was higher compared to after SEMS

394   (1.57±0.14 pre-SEMS vs 1.35±0.32 post-SEMS, p=0.02, Mean±SD). The S50 parameter before

395   SEMS was also significantly higher than after SEMS (1.21±0.06 pre-SEMS vs 0.99±0.21 post-

396   SEMS, p=0.02, Mean±SD). However, H-max, b, Slope at 50% H-max and Intensity Range are not

397   significantly different before and after SEMS.

398

399   Table 2. Descriptive Statistics of Parameters for H Reflex by Pre and Post SEMS

Parameter	Pre N=18 Mean±SD Median (range)	Post N=18 Mean±SD Median (range)	Change (Post-Pre) N=19 Mean±SD Median (range)	P-value[1]
H-max	99.9±4.2 98.7 (94.52,107.55)	98.4±3.5 98.2 (92.46,104.60)	-1.5±3.7 -0.8 (-10.23, 5.60)	0.26
b (Steepness)	0.1±0.02 0.1 (0.04, 0.11)	0.1±0.02 0.1 (0.04,0.11)	-0.01±0.02 -0.01 (-0.05,0.02)	0.24
Hslope (Slope at 50% H-max)	352.7±105.1 338.6(216.07, 616.72)	397.2±108.3 381.4(221.45, 582.10)	44.5±108.2 36.05 (-167.28,305.21)	0.29
Intensity corresponding to H-max (x MT)	1.6±0.1 1.56 (1.40,1.83)	1.4±0.32 1.5 (0.77, 1.78)	-0.2±0.3 -0.2 (-0.72, 0.18)	0.02
Intensity range (Max - Min) (x MT)	0.6±0.14 0.56 (0.40, 0.83)	0.6±0.2 0.6 (0.21, 0.90)	-0.02±0.1 -0.02(-0.35, 0.26)	0.30
S_{50} (Intensity corresponding to 50% H-max) (x MT)	1.2±0.06 1.2 (1.11, 1.37)	1.0±0.2 1.1 (0.54, 1.29)	-0.2±0.2 -0.1 (-0.62, 0.10)	0.02

400   [1]Testing the significance of pre-post change based on F-tests of rmANOVA.

401

402   Table 3. Correlations between parameter estimates of H-reflex and M-wave

Parameter	Correlation Coefficient r (p-value) Pre	Post	Change
Normalized M-Max and H-Max	-0.09 (0.72)	-0.12 (0.62)	0.46 (0.06)
B (Steepness)	-0.05 (0.84)	0.25 (0.32)	0.08 (0.74)
Hslope and Mslope (at 50% of Hmax and Mmax)	-0.07 (0.7810)	0.27 (0.2838)	-0.09 (0.71)
Intensity corresponding to M-max and H-max (x MT)	-0.24 (0.3338)	0.31 (0.2047)	0.75 (0.0004)
Intensity range (Max - Min) (x MT)	-0.24 (0.3338)	0.13 (0.6198)	0.41 (0.09)
S_{50} (Intensity corresponding to 50%) (x MT)	-0.17 (0.50)	0.47 (0.047)	0.70 (0.001)

403

404   In order to understand whether SEMS induced changes in H-reflex and in M-wave are comparable,

405   we have also analyzed correlation between SEMS-induced changes in the parameters of H-reflex

406   and M-wave recruitment curves. As shown in Table 3, the SEMS-induced changes in the intensity

407   corresponding to M-max and H-max, as well as S50 parameters were significantly correlated for

408   M-wave and H-reflex responses (both r>0.07, p <0.001). However, important to note that Hslope

409   and Mslope at 50% of max and steepness of the curves were not correlated (r=0.09, p=0.71 and

410   r=0.08, p=0.74 respectively).

411

412   Modulation of H-reflex by SEMS train is long-lasting and sustained after stop

413   of SEMS

414   We have also examined whether the effect of SEMS are long-lasting, i.e. how long the

415   observed threshold changes were sustained by measuring H-reflex before, immediately after

416   SEMS and after 1 hour post stopping of SEMS (Fig 6). The threshold intensity required for H

417   reflex was still significantly lower after 1 hour post SEMS administration;79.8 ± 3.3 % post-SEMS

418   compared to 100% pre-SEMS (n=4; p<0.05). Importantly, the decrease in the MT intensity

419   following SEMS application, however, was not associated with the changes in Hmax. Hmax

420   amplitude was 1.96 ± 0.19 mV before and 1.91 ± 0.12 mV after SEMS application (n = 8, p =

421   0.156).

422   **Figure 6.   Long lasting effect of SEMS on the H-reflex.** Summary of results demonstrating

423   change in the stimulation current intensity required to elicit the minimal H-reflex after SEMS

424   administration for all participants. Summary of results are presented as percent change of control

425 for each participant, where control (before SEMS) represent 100%. Effect of SEMS in decreasing

426 threshold intensity lasts for at least 1 hour after stop of SEMS.

427

Effects of SEMS on TMS evoked responses

429 We have also examined whether SEMS administration may affect TMS induced responses.

430 We recorded TMS evoked responses from hand FDI muscle before and after SEMS administration.

431 Representative traces recorded from FDI muscle before and after SEMS in the same participant

432 presented in Fig 7A. We did not observe any changes in TMS evoked responses after SEMS.

433 Percentage change of TMS evoked MEP responses is presented in Fig 7B. There was no significant

434 change in the amplitude of MEP recorded from FDI muscles after SEMS i.e. 102.6 ± 24.1 % (n=8;

435 $p > 0.05$). These results suggest that an observed effect of SEMS on H-reflex and M wave

436 responses are local and do not involve supraspinal systems.

437 **Figure 7. Repeated SEMS administration does not affect the TMS-evoked responses**

438 **recorded from hand muscles.** (A) Representative TMS evoked motor evoked potentials (MEP)

439 recorded from first dorsal interosseous (FDI) muscle from one participant before and after SEMS

440 administration. (B) Summary of results demonstrating no change in the amplitude of MEPs

441 recorded from FDI muscles in response to TMS after SEMS administration. Results are presented

442 as percent change of control for each participant. Diagram illustrating the position of TMS

443 stimulation coil and recording electrodes.

444

Discussion

There are two major outcomes from this study. (1) Parameters for SEMS stimulation and characteristics of CMAP responses recorded simultaneously from several leg muscles have been established; results demonstrate that CMAP responses measured from different muscles in legs and evoked by single-pulse SEMS applied at different spinal levels can serve as an feasible approach for the neurophysiological evaluation of neural pathways innervating leg muscles. (2) SEMS applied as a train of repetitive pulses may modulate the amplitude of both the direct (M-wave) and reflex (H-reflex) responses recorded from SOL, MG and LG leg muscles. No previous study has compared effects of repetitive SEMS on M- and H- responses of these three sections of the triceps surae muscle group. Together these results suggest that SEMS may become a novel non-invasive and painless tool to induce long-lasting plasticity of the lower limb spino-neuromuscular circuitry.

SEMS applied at different spinal levels is an effective non-invasive stimulation protocol for activation of distinct leg muscles

Electro-magnetic stimulation applied at the periphery i.e. over spinal roots, nerves or muscles has been recently utilized in clinical research as a new painless and noninvasive approach to deliver electrical excitation [13, 29]. Neurophysiological parameters obtained by measures of CMAP responses evoked by lumbosacral electro-magnetic stimulation are comparable with those obtained with electrical motor-root stimulation using surface electrodes [10, 38], or by inserting needle electrodes into the tissues around the lumbosacral motor roots [39, 40]. However, these

466   types of electrical stimulation often associated with pain, discomfort, and sensory disturbance, in

467   contrast to non-invasive and painless SEMS application. Systematic examination and comparison

468   of CMAP responses recorded in different leg muscles and evoked by administration of SEMS at

469   different spinal levels in same participant is understudied.

470   Several previous reports describe neurophysiological characteristics of CMAPs responses

471   evoked by SEMS. For example, positioning of the coil over the C7 spine was optimal for hand

472   muscle stimulation [41] and SEMS administration over thoracic spinal levels induced activation

473   of leg muscles [13, 14]. Applied at lumbosacral level it was used for estimates of peripheral motor

474   nerve conduction and diagnosis of lumbosacral motor radiculopathy [42].

475   Our current study examined and described the properties of CMAP responses recorded

476   simultaneously from several muscles (soleus, MG, LG, BF, ST and VL) in both legs and evoked

477   by positioning of electro-magnetic coil at different spinal levels (T12 to S1) using stimulus of

478   various intensity (40% to 80% of coil maximum intensity). Our results demonstrate that SEMS

479   administration at different spinal levels result in activation of distinct muscles with different

480   CMAP properties. CMAP responses recorded from the same muscles were showing different

481   latencies and different amplitudes depending on the spinal level where SEMS was administered.

482   Presented results demonstrate that SEMS is an effective non-invasive tool that can activate distinct

483   pathways and diverse leg muscles when applied at different spinal levels. Together these results

484   indicate that SEMS is an appropriate approach for neurophysiological evaluation of conduction at

485   neural pathways innervating leg muscles. The measures of amplitude and latency of CMAP

486   responses recorded from various muscles in healthy individuals may serve as a reference for future

487   studies examining effects of SEMS in SCI individuals or other neurological disorders.

488 **SEMS applied as repetitive trains is an effective tool to modulate H-reflex and**

489 **M-response**

490   Another important finding was that SEMS applied as repetitive train induced modulation

491 of electrically evoked M-response and H-reflex in the SOL, MG, LG muscles. The H-reflex which

492 is a response elicited by electrical stimulation of afferent (Ia sensory) fibers travels to the

493 motoneurons pool of the corresponding muscle in the spinal cord with subsequent monosynaptic

494 recruitment of alpha motor neurons and then through the efferent (motor) fibers to the muscle

495 spindles [6, 43]. H-reflex and muscle M-response evoked in the SOL muscle by the tibial nerve

496 electrical stimulation are recognized as standard tools for studying the integrity and plasticity at

497 spino-muscular circuitry [44, 45]. Any change in H-reflex amplitude under a given condition

498 reflects changes in the excitability of the afferent/motoneuron/efferent reflex pathways, while

499 changes in M-wave amplitude reflect changes in excitability in the efferent fibers only.

500   In this study we have examined effect of SEMS on the amplitude of M- and H-responses

501 recorded simultaneously from SOL, MG and LG muscles. Although all these three muscles belong

502 to triceps surae muscle group, these muscles differ in function, composition, and innervation [45].

503 Comparison of the effects of SEMS on M- and H- responses of these muscles have not been

504 described by other groups.

505   Our results demonstrate that application of SEMS for 30 mins at 0.2Hz frequency induced

506 substantial facilitation of both M-response and H-reflex in all three SOL, MG and LG muscles. To

507 better characterize the SEMS-induced modulation of M- and H-responses we have analyzed the

508 effects of SEMS on the recruitment curves that have been obtained from SOL muscles. Results

509 suggest that effects of repetitive SEMS associated with the significant leftward shift of the

510 recruitment curves for both M- and H-responses. Effects of SEMS on H-reflex and M-wave were

511 associated with a marked decrease in the threshold currents to evoke H- and M-responses, with no

512 change in latencies of these responses. This effect of SEMS lasted for at least 1 hour after stopping

513 of SEMS. The leftward shift of the recruitment curve for H-reflex and the decrease in MT current

514 intensity to evoke the H-reflex after SEMS application was not associated with changes in the

515 amplitude of Hmax (Figs. 4, 5), known as an estimate of the number of motor neurons (MNs)s that

516 could be activated [46, 47]. Although the mechanisms underlying effects of repetitive SEMS on

517 neural plasticity are yet to be discovered, SEMS-induced a leftward shift of the recruitment curve

518 and a decrease of the MT for both M- and H- responses clearly indicate that prolonged (30 min)

519 application of pulsed SEMS at low (0.2 Hz) frequency induces substantial changes in the

520 excitability of spino-muscular circuitries.

521

522 **Comparison of the effects of SEMS with other types of stimulation to modulate**

523 **H-reflex and M-response**

524 Our results of the neuromodulation of M-wave and H-reflex responses induced by non-

525 invasive SEMS application is similar to results reported by other types of stimulation techniques.

526 For example similar modulation of M-wave and H-reflex responses was reported with

527 transcutaneous direct current stimulation (tsDCS), where tsDCS paired with transcranial magnetic

528 stimulation TMS induced substantial reduction in the motor threshold for both M- and H-

529 responses [48]. Applied alone, tsDCS was found to modulate characteristics of M-wave and H-

530 reflex as well, with type of modulation depending on applied current polarity, i.e. anodal or

531 cathodal current application [49-51]. This type of neuromodulation has been attributed to changes

532 in the efficacy of the Ia fiber-motoneuron synapse [52, 53], or excitability at spino-neuromuscular

533 circuitry [51]. Modulation of synaptic transmission within the spinal cord may involve changes in

534 several neural properties, including changes in membrane potential with a corresponding changes

535 in synaptic activity [54, 55] and/or modulation of neuronal firing rate [56, 57]. Several reports

536 suggest the primary role of modulation of axonal excitability in the effects of tsDCS [51, 58, 59].

537 Effect of tsDCS on axonal excitability was suggested to be attributed to depolarizing current

538 interaction with axonal voltage-gated Na+ channels [60, 61]. Long-lasting effects of tcDCS on H-

539 reflex has been suggested to be a result of triggering a self-sustained opening of 'persistent' Na+

540 channels [51], known to be able to keep the membrane potential steadily depolarized [62] and alter

541 the distribution of proteins on the plasmatic membrane [63].

542 SEMS and established electrical stimulation techniques may, however, act differently on

543 neural plasticity and have different effects within spino-muscular circuits. In contrast to electrical

544 stimulation, it has been reported that SEMS activates deep conductive structures, produces strong

545 muscle contractions and recruits proprioceptive afferents with minimal cutaneous recruitment of

546 sensory fibers [64-66]. While there are some differences between SEMS and tcDCS induced

547 neuromodulations we do not exclude a possibility that these cellular mechanisms suggested to

548 describe the modulatory effects of the tcDCS may be involved in the reporting here neuroplasticity

549 induced by non-invasive SEMS. Although the intrinsic processes underlying the effects of SEMS

550 on M- and H-responses reported in this study require further examination, our results strongly

551 suggest that SEMS is an effective and non-invasive approach to modulate function in spino-

552 neuromuscular circuitry.

553 Results of our analyses demonstrate strong correlation of some parameters (i.e. Normalized

554 Mmax and Hmax; Intensity corresponding to Mmax and Hmax; S50; intensity corresponding to

555   50% of max response), but lack of correlation of other parameters of H-reflex and M-wave

556   recruitment curves such as steepness of the fitted recruitment curves, intensity range and changes

557   of H-slope and M-slope at 50% of Hmax and Mmax. These observations suggest that SEMS may

558   exert its modulatory action at the level of (i) spinal neural networks, including efficacy of synaptic

559   transmission and/or neuron membrane potentials, or (ii) modulating properties of peripheral nerve,

560   i.e. excitability of the afferent sensory and/or efferent motor axons, or (iii) occurs at both spinal

561   and peripheral levels. Experiments using animal models are required to determine the mechanisms

562   that underlay the SEMS induced neuroplasticity and these experiments are on-going.

563   In conclusion, reported here for the first time the ability of the SEMS to exert the long-

564   lasting modulation of excitability at spino-neuromuscular circuitry. suggests a potential

565   application of SEMS in clinics for number of spinal or peripheral nerve conditions. Thus

566   modulating the strength of the motor and sensory inputs could lead to a change of the H-reflex

567   characteristics [67]. SEMS could be also used for improving the diminished axonal conduction as

568   is the case of many clinical neurological disorders [68], including SCI [2, 3], as it has been

569   demonstrated using animal SCI models [1, 4, 22].

570

573

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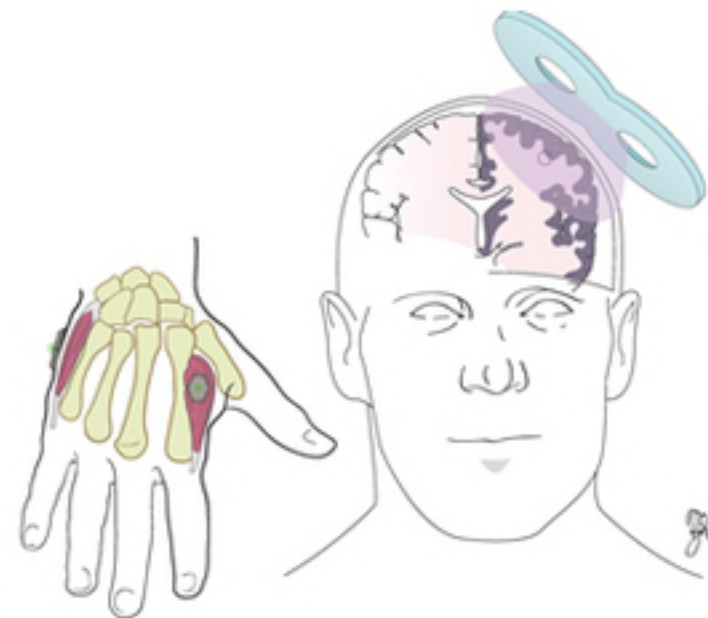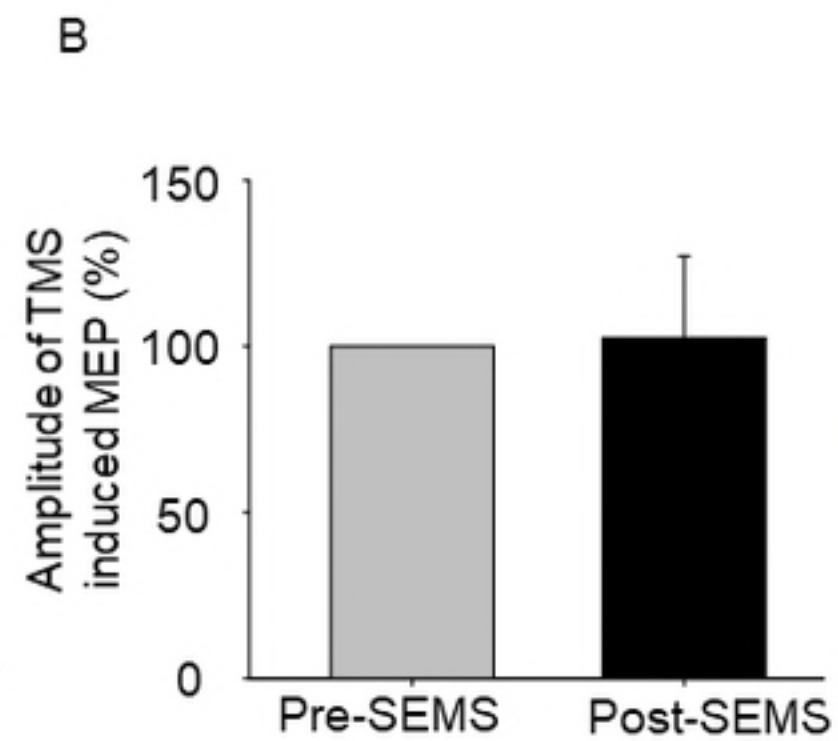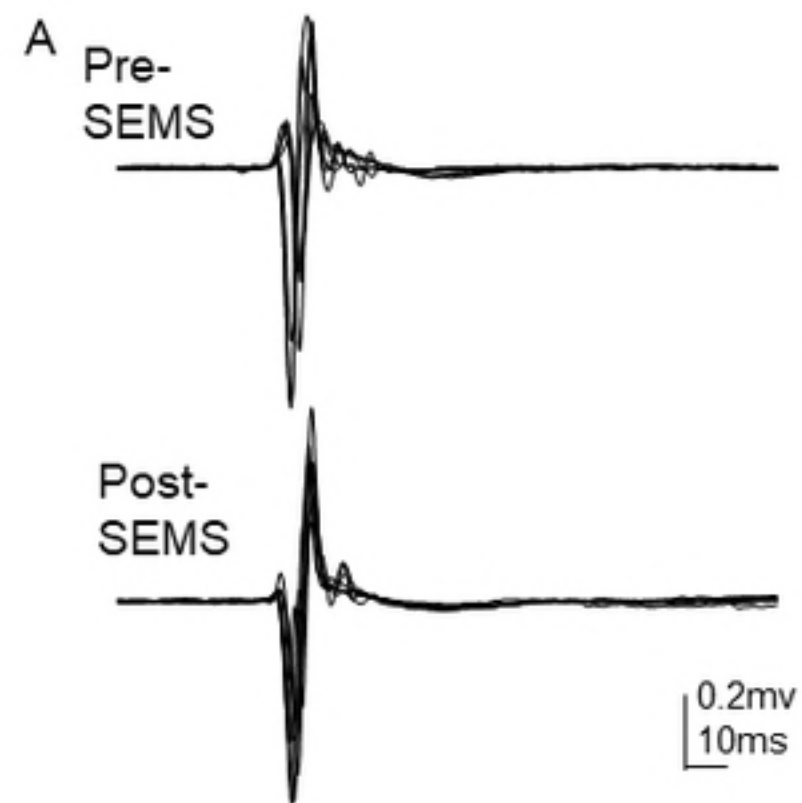
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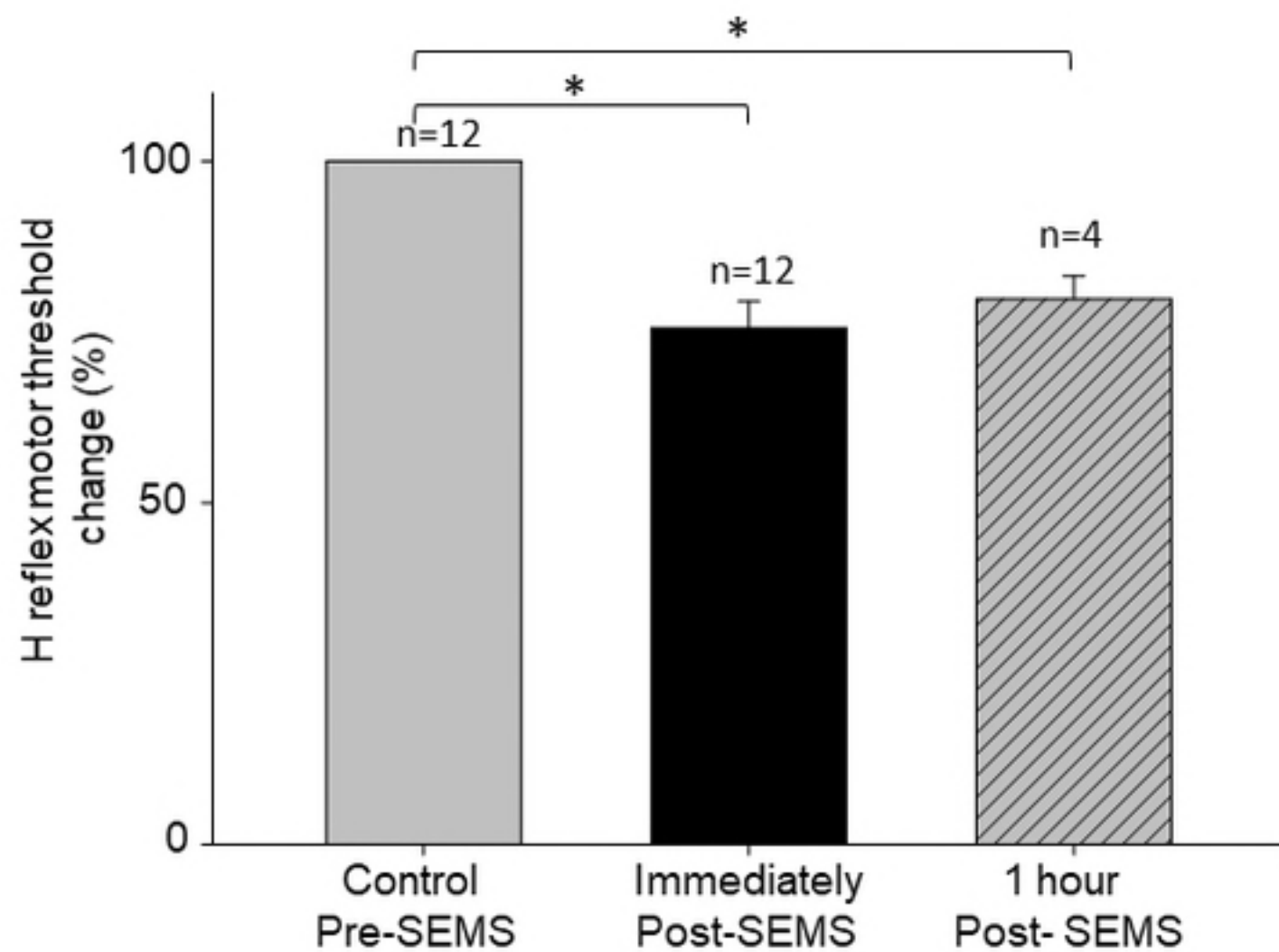
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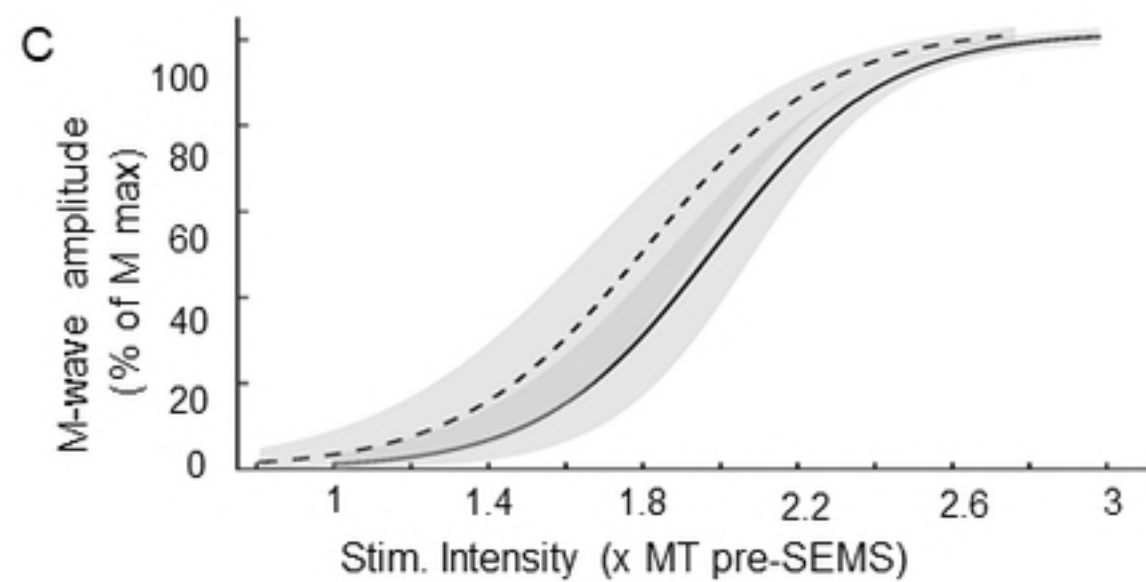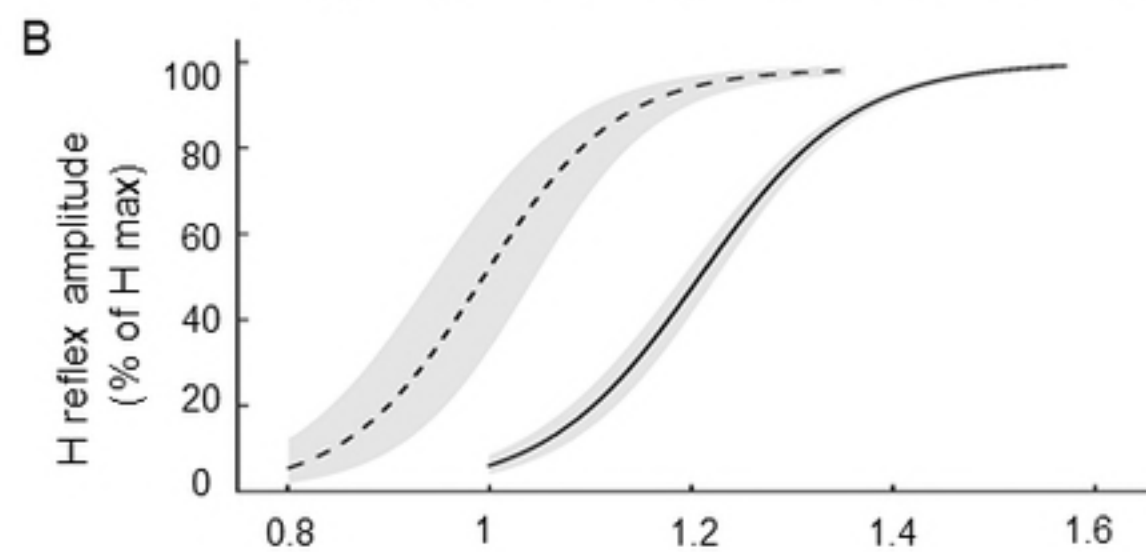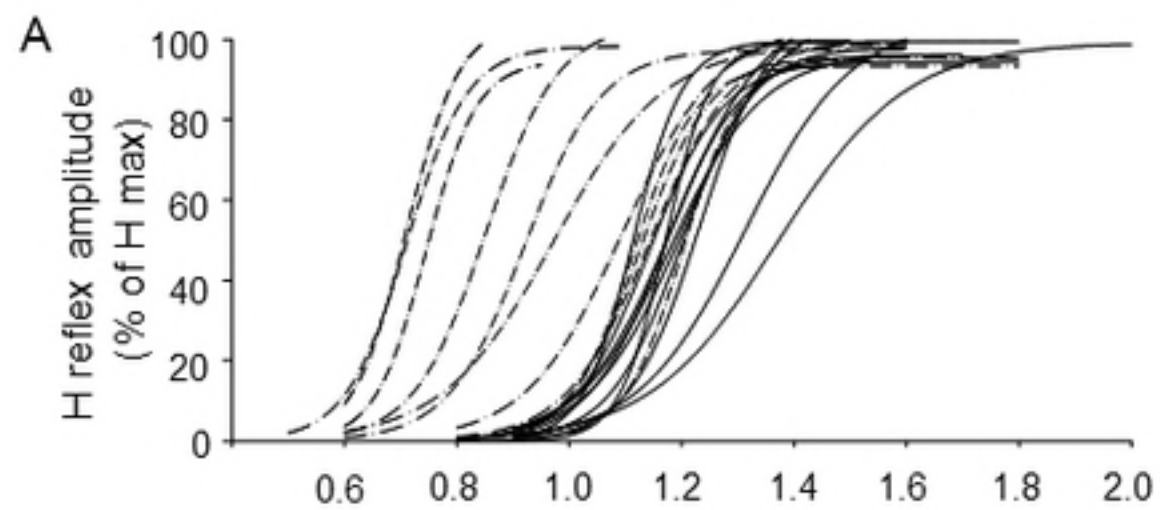
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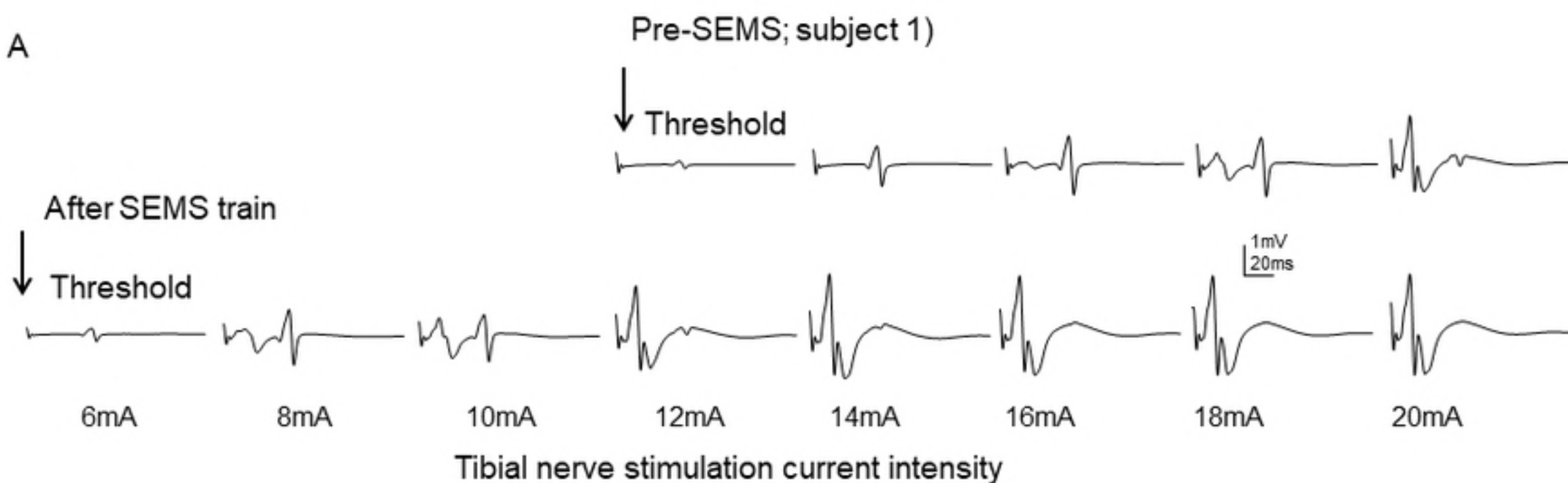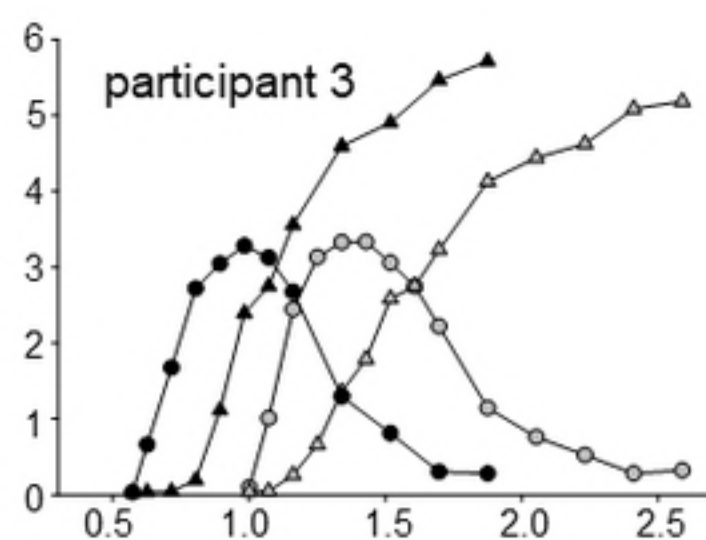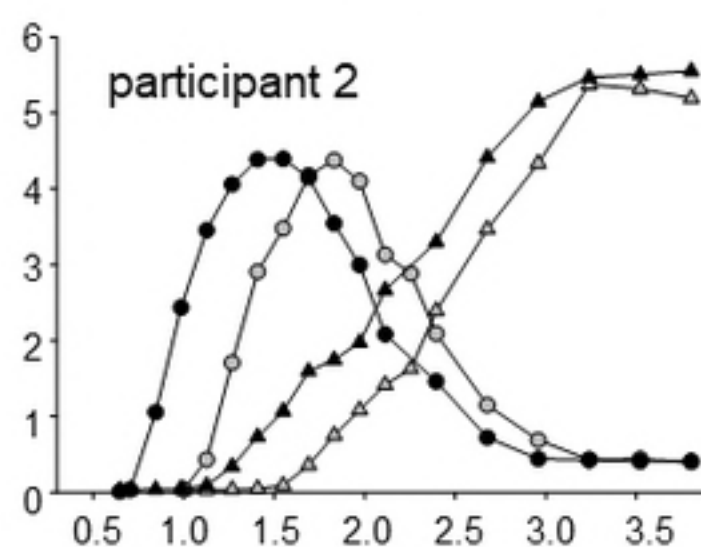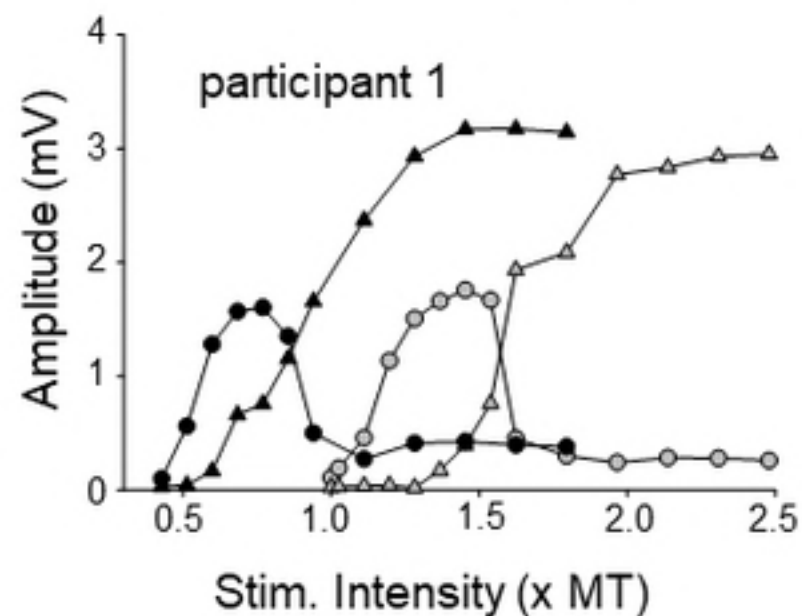
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A

Pre-SEMS

Post-SEMS

0.2mv
10ms

B

A

Pre-SEMS; subject 1)

↓ Threshold

After SEMS train

↓ Threshold

1mV
20ms

6mA   8mA   10mA   12mA   14mA   16mA   18mA   20mA

Tibial nerve stimulation current intensity

B
△ H-reflex (Pre-SEMS)   ▲ H-reflex (Post-SEMS)
● M-wave (Pre-SEMS)   ● M-wave (Post-SEMS)

participant 1

participant 2

participant 3

Amplitude (mV)

Stim. Intensity (x MT)

A   Pre - SEMS

SOL

MG

LG

B   Post - SEMS

SOL

MG

LG

0.2mV
20ms

SEMS Stim.

VL

BF

ST

SOL

LG

MG

b) SEMS at T12-L1 Spinal level

c) SEMS at L2-L5 Spinal level

0.2mV
20 ms