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1 **EFFECT OF GAIT IMAGERY TASKS ON LOWER LIMB MUSCLE**
2 **ACTIVITY WITH RESPECT TO BODY POSTURE**

3

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16 *Summary. (max 180 words)–*

17 The objective of this study was to evaluate the effect of gait imagery tasks on lower limb muscle
18 activity with respect to body posture. The sitting and standing position and lower limb muscle activity
19 was evaluated in 27 healthy female students (24.4 ± 1.3 yrs, 167.2 ± 5.2 cm, 60.10 ± 6.4 kg). Surface
20 electromyography was assessed during rest and in three different experimental conditions using
21 mental imagery. These included; a rhythmic gait, rhythmic gait simultaneously with observation of a
22 model and rhythmic gait after performing rhythmic gait. The normalized rmsEMG values with respect
23 to corresponding rest position were compared using non-parametric statistics. Standing gait imagery
24 tasks had facilitatory effect on proximal lower limb muscle activity. However, EMG activity of distal
25 leg muscles decreased for all gait imagery tasks in the sitting position, when the proprioceptive
26 feedback was less appropriate. For subsequent gait motor imagery tasks the muscle activity decreased,
27 probably as result of habituation. In conclusion the effect of motor imagery on muscle activity appears
28 to depend on relative strength of facilitatory and inhibitory inputs.

29

30 **Keywords:** gait, motor imagery, surface electromyography

31

32

33 Motor imagery (MI) represents a pure cognitive process, which positively influences motor
34 performance in healthy subjects which has been shown for sport performance, e.g. gymnastics, ballet
35 and tennis (Guillot, Di Rienzo, Macintyre, Moran, & Collet, 2012). In addition this has been shown
36 in patients following motor impairment and has been used in physical therapy during recovery of
37 function (Lotze & Halsband, 2006; Mizuguchi et al., 2012). Specifically walking skills in
38 neurological patients improved after motor imagery exercise (Dunsky, Dickstein, Marcovitz, Levy,
39 & Deutsch, 2008; Oostra, Oomen, Vanderstraeten, & Vingerhoets, 2015). Home-based motor
40 imagery gait training programs have been shown to improve gait parameters including; walking
41 speed, stride length, cadence, single and double support time in chronic post-stroke subjects (Dunsky,
42 Dickstein, Marcovitz, Levy, & Deutsch, 2008). Motor imagery training includes imagery of walking
43 tasks in combination with physical therapy has been suggested to be more effective for improving
44 gait velocity in sub-acute stroke patients then physical therapy alone (Oostra, Oomen, Vanderstraeten,
45 & Vingerhoets, 2015). In addition videotape-based locomotor imagery training together with regular
46 physical therapy has been shown to improve walking ability in post-stroke and people with
47 Parkinson's disease more than gait training alone (El-Wishy & Fayez, 2013; Hwang et al., 2010).

48 Motor imagery can be described as a conscious mental simulation of an action without actual
49 execution, is accompanied by activity in specific neural substrates (both supraspinal and spinal)
50 similar to those involved in the actual executed movement. Meta-analysis on effect of motor imagery
51 on brain structures conducted by Hetu, *et al.* (2013) provided evidence that motor imagery activates
52 motor related brain networks including large fronto-parietal and subcortical regions involved in motor
53 execution. Several studies provided evidence that motor imagery increases excitability in
54 corticospinal tracts which projects directly to motoneurons and their interneurons controlling the
55 muscles (Clark, Mahato, Nakazawa, Law, & Thomas, 2014; Cowley, Clark, & Ploutz-Snyder, 2008;
56 Oku, Ishida, Okada, & Hiraoka, 2011; Roosink & Zijdwind, 2010). This has been shown to increase
57 the excitability of spinal reflexes (Li, Kamper, Stevens, & Rymer, 2004) and also in muscle
58 proprioceptive structures (muscle spindle Ia afferent fibers) (Bonnet, Decety, Jeannerod, & Requin,
59 1997). So it seems that the motoneuron pool of muscle involved in imaginary movement receives
60 summation of neural inputs via descending and ascending neural pathways in similar way as during
61 real movement. The possibility that mental imagery can have an effect on the muscles that create the
62 movement is supported by the positive influence of motor imagery training on muscle strength (Clark
63 et al., 2014; Yue & Cole, 1992). However the influence of motor imagery on electromyography
64 (EMG) measures is not clear yet. To date several studies have found no significant effect of motor
65 imagery on electromyographic activity during imaginary pointing arm movement for upper limb
66 muscles (Demougeot & Papaxanthis, 2011; Gentili, Papaxanthis, & Pozzo, 2006) during imaginary
67 pointing arm movement for upper limb muscles including anterior deltoid, triceps and biceps brachii,

68 pectoralis major. In addition, Ranganathan, Siemionow, Liu, Sahgal, & Yue (2004) found no increase
69 in activity of biceps brachii and finger abductor during imaginary isometric little finger abduction
70 and elbow flexion, and Lemos, Rodrigues, & Vargas (2014) who found no increase in activity of the
71 gastrocnemius lateralis during imaginary rising on tiptoes. However, Oku, Ishida, Okada, & Hiraoka
72 (2011) found increased EMG in extensor carpi radialis activity during imaginary wrist extension and
73 Guillot et al. (2007) and Dickstein, Gazit-Grunwald, Plax, Dunsky, & Marcovitz (2005) showed
74 increased EMG activity of nine upper limb muscles in agonists, synergists, fixators and antagonists
75 during imaginary lifting a weighted dumbbell and increased EMG activity of gastrocnemius medialis
76 and rectus femoris when performing imaginary rising on tiptoes respectively.

77 Surface electromyographic measurements reflect, to some extent, the effort of neural system
78 for movement execution as EMG signal is usually proportional to the level of motor unit activity
79 (Richards, 2008). The muscle activity is altered by variations in the balance between inhibitory and
80 facilitatory input which go in parallel to the motoneuron pool, the terminal part of spinal afferent or
81 efferent sensory/motor pathways (Daroff et al., 2012). So it might accepted that even during MI the
82 magnitude of EMG activity reflects the summation of facilitory and inhibitory inputs. This
83 assumption is supported by recent findings, which had shown that the increase of EMG activity during
84 MI mirrors a number of facilitatory inputs including mental effort related to e.g. characteristics of
85 imagined object, the heavier was the object lifted in imagination the showed a greater EMG signal
86 during MI (Bakker, Boschker, & Chung, 1996) and tends to be more pronounced in complex
87 functional movements (Bakker et al., 1996; Guillot et al., 2012; Guillot et al., 2007). The EMG signal
88 during motor imagery is classified mostly as subliminal (Guillot et al., 2012; Guillot et al., 2007) or
89 background muscle activity (Oku, Ishida, Okada, & Hiraoka, 2011) which indicates that detectable
90 muscle activity during MI does not have comparable magnitude and phasic pattern to real movement
91 execution. As the amount of increase in EMG amplitude during motor imagery is positively correlated
92 with the amount of corticospinal excitability (Oku, Ishida, Okada, & Hiraoka, 2011) and with respect
93 to previous findings that corticospinal excitability and brain activity during motor imagery is
94 enhanced with the real sensory feedback generated by holding an object which is imaginary
95 manipulated (Mizuguchi et al., 2012) we speculate that EMG activity during gait imagery may be
96 influenced by character of sensory feedback with respect to sitting (non-default position for walking)
97 or standing (default position for walking) body position during imagination.

98 With respect to imaginary training protocols in sport or in rehabilitation it has been suggested
99 that simultaneously observing somebody doing the task during motor imagery further positively
100 influences neural activity and enhances motor learning processes (Nedelko, Hassa, Hamzei,
101 Schoenfeld, & Dettmers, 2012; Roosink & Zijdewind, 2010; Wright, Williams, & Holmes, 2014). In
102 similar way with respect to motor learning even previous practice of imaginary movement facilitates

103 neural activity more than imagery before practice, improves imagination ability of this movement
104 (Wriessnegger, Steyrl, Koschutnig, & Muller-Putz, 2014) and combination of imagination with real
105 practice is more effective for motor recovery than movement imagination or execution alone.
106 Therefore the simultaneous observing of imaginary movement will have facilitatory effect on muscle
107 activity.

108 It has also been previously suggested that the effectivity of the motor imagery training depends
109 on individual's imaging ability (Gregg, Hall, & Butler, 2010). Subjects with a good motor imagery
110 ability show a greater performance improvement following motor imagery training than do subjects
111 with a poor imagery ability (Mizuguchi, Yamagishi, Nakata, & Kanosue, 2015).

112 The aim of the present study was to analyze the effect of gait imagery tasks from the first
113 person perspective on both proximal and distal lower limb muscle activity. Based on the prior finding
114 that motor imagery activates neural structures in similar way as movement execution and that muscle
115 activity reflects the summation of neural inputs coming to motoneuron pool via afferent and efferent
116 pathways we hypothesized that: (1) imagination of gait (which is considered as complex functional
117 task) modulates lower limb muscle activity, (2) the magnitude of muscle activity reflects character of
118 peripheral sensory inflow during imagination with respect to body posture and (3) the magnitude of
119 muscle activity is further influenced with respect to additional cognitive and motor task.

120 Therefore this study aimed to evaluate the electromyographic activity of proximal and distal
121 lower limb muscles, which participate synergically on gait execution, during gait imagery tasks
122 compare to rest conditions. This would potentially further our understanding of influence of gait
123 imagery task on motor system and the effect of imagining or observing gait activity of lower limb
124 muscles. This in turn provides important information for gait imagery rehabilitation protocols and
125 could increase our understanding of gait control mechanisms.

126

127 Method

128 *Participants*

129 Twenty seven healthy females participated in this study. Their mean (\pm SD) age, height and
130 weight were 24.4 ± 1.3 yrs, 167.2 ± 5.2 cm and 60.10 ± 6.4 kg. All participants were recruited from
131 students from a Physiotherapy department of Palacky University. All participants had good cognitive
132 function and communicative skills necessary for motor imagery and were able to generate gait motor
133 imagery. Only participants with at least moderate visual and kinesthetic imagery ability, evaluated by
134 Revised Movement Imagery Questionnaire (MIQ-R), were included in the study (Smith & Collins,
135 2004). MIQ-R represents a reliable tool to assess motor imagery ability in healthy persons. MIQ-R
136 consists of an eight-item self-report questionnaire using two 7-point scales to evaluate ability to form
137 visual and kinaesthetic mental images (Hall & Martin, 1997). The exclusion criteria included

138 psychiatric, neurological or musculoskeletal disorders, balance or walking problems, the use of a
139 walking aid, chronic pain, pregnancy, the use of medication affecting the level of vigilance and
140 uncorrected visual impairments. The dominant lower limb was the right side in all participants,
141 determined as preference for kicking a ball (Seeley, Umberger, & Shapiro, 2008). Testing occurred
142 in a quiet room in one day. All participants signed an informed consent prior to participating in this
143 study. The procedures, which were approved by the local ethics committee, were performed
144 according to the ethical standards of the Declaration of Helsinki.

145 *Motor imagery ability measures*

146 When completing the MIQ-R, participants are asked to perform one of four movement tasks
147 and then rate the ease with which they form visual and kinaesthetic images of this movement (from
148 1 = “very hard to see/feel” to 7 = “very easy to see/feel”). In the study mean MIQ-R scores (SD) were
149 47.7 (5.9) for both subscales, 24.15 (2.94) for the visual subscale, and 23.15 (3.15) for the kinaesthetic
150 subscale. The MIQ-R has demonstrated adequate internal consistency with Cronbach α coefficients
151 0.78 and 0.76 for visual and kinaesthetic subscales respectively. MIQ-R mean scores and consistency
152 were comparable to those observed in previous MI studies (Hall & Martin, 1997; Guillot, et al., 2012).

153 *Electromyography measures*

154 Muscle activity was measured using surface EMG using two self-adhesive electrodes (Ag-
155 AgCl). The electrodes were placed in parallel to the muscle fibers in the midline over the muscle belly
156 with an inter electrode distance of 2 cm. Prior to placing the EMG surface electrodes, the skin was
157 abraded and cleaned. EMG activity was recorded from biarticular lower limb muscles involved with
158 gait execution by synergistic action (Chvatal & Ting, 2012). Three distal muscles of the dominant
159 lower limb: tibialis anterior (TA), gastrocnemius lateralis (GL), gastrocnemius medialis (GM), and
160 three proximal muscles of the dominant lower limb: biceps femoris (BF), semitendinosus (ST) and
161 rectus femoris (RF) were measured. The reference electrode was placed over the fibula head. EMG
162 data were recorded at 1000 Hz using the wireless system TeleMyo 2400T G2 (Noraxon Co., USA)
163 with a system bandwidth was 20-1000 Hz. Real-time EMG signals were sent via telemetry at 1,000
164 Hz to an A-D converter (Noraxon Co., USA). The raw EMG signals were full wave rectified and the
165 root mean squared value of EMG (rmsEMG) signals was calculated using a time averaging period of
166 25 ms (Guillot, et al., 2007). The processing of the signal was performed by using the software
167 MyoResearch XP Master Edition 1.08.17 (Noraxon Co., USA). Raw EMG signal was visually
168 checked prior to processing and analysis to verify the absence of any artifacts.

169 *Procedure*

170 The test protocol was conducted with respect to previous findings such that the imagination
171 ability was enhanced when imagination was done from first person perspective, and is performed

172 with externally given auditive feedback (Guillot, *et al.*, 2007; Heremans, *et al.*, 2012; Koehler, *et al.*,
173 2012; Mizuguchi, *et al.*, 2012; Roosink & Zijdewind, 2010).

174 EMG data were initially collected in two default rest positions, sitting (non-default position
175 for walking) and standing (default position for walking) without performing any voluntary activity or
176 motor imagery, and then within six motor imagery experimental conditions in the following order:

- 177 1. gait imagery in the sitting position, gait imagery in the standing position,
- 178 2. gait imagery and simultaneous gait observation in the sitting position, gait imagery and
179 simultaneous gait observation in the standing position,
- 180 3. gait imagery in the sitting position after gait execution, gait imagery in the standing position
181 after gait execution.

182 Experimental conditions are illustratively demonstrated in Figure 1.

183 FIGURE 1

184 *Figure 1 insert here*

185 Default sitting or standing positions were standardized for all experimental conditions. In the
186 sitting position, the participants were seated upright in a chair that leaned against the back and arm
187 rest. In the standing position, the participants were standing upright with hands along their body. In
188 both default positions, the feet were placed a pelvic width apart. In all experimental situations, the
189 position of the feet was unchanged.

190 For every participant and for all tested conditions, the rhythm of gait was given to the
191 participants using a metronome set at 110 beats per minute, to replicate a normal gait cadence All
192 tested participants reported that they were able to imagine gait well at this step frequency. In the first
193 experimental imaginary gait conditions for sitting and standing, the participants were instructed to
194 imagine a rhythmic gait as vividly as possible, in the first person perspective, the instruction was
195 “*Imagine yourself walking on the pace of the metronome*“ without making any actual movements. In
196 second tested conditions, the participants observed the rhythmic gait of a second person in frontal
197 plane from posterior side on the projection screen (200 x 200 cm) placed 2 meters in front of them.
198 The participants were instructed to watch the gait and to simultaneously imagine a rhythmic gait as
199 if they were walking (the instruction was “*Observe the woman on the screen walking at the pace of*
200 *the metronome and simultaneously imagine yourself walking at the same pace*“). Next, real rhythmic
201 walking at the pace of the metronome in hospital corridor was performed by the participants for a few
202 minutes to enhance further rhythmic gait imagination ability (Wriessnegger, Steyrl, Koschutnig, &
203 Muller-Putz, 2014). Just after real rhythmic walking, third experimental conditions were performed,
204 the instruction within the gait imagery task after gait execution was the same task as in the first
205 experimental conditions “*Imagine yourself walking on the pace of the metronome*“. Each gait imagery

206 task lasted for approximately 60 seconds. None of participants mentioned feelings of fatigue during
207 the experimental session.

208

209 *Data processing*

210 The rmsEMG [%] was calculated for every experimental condition in sitting or standing position and
211 then normalized to the rmsEMG of default sitting or standing rest positions. For the rest sitting and
212 standing positions the average rmsEMG values of all tested the muscles were calculated over a 20
213 seconds interval. These values calculated during the rest condition without any motor imagery were
214 considered as reference values. For all rhythmic gait imagery tasks the mean rmsEMG values were
215 calculated over six gait cycles for the dominant lower limb. The duration of evaluated EMG period
216 was 6.6 seconds which was calculated from the metronome frequency where one gait cycle was 1.1
217 seconds. This period was selected from the middle part of the measured data for every experimental
218 condition with respect to adaptation on the imagery task. The mean rmsEMG values during
219 experimental gait imagery tasks were expressed as a percentage of reference value. Gait imagery
220 experimental tasks conducted in sitting position were normalized to the respective reference value
221 obtained in rest sitting position and gait imagery tasks conducted in standing position were
222 normalized to the respective reference value obtained in rest standing position for every participant
223 and tested muscle.

224

225 *Statistical analysis*

226 Data were tested to determine if they were normally distributed using Kolmogorov-Smirnov
227 test. All data were found not to be normally distributed, ($p < 0.05$), therefore non-parametric tests were
228 used throughout the analysis. For the statistical analysis the non-parametric Wilcoxon signed-rank
229 test was performed with the alpha value was set at $p < 0.05$. This allowed the comparison of the
230 reference values for sitting and standing positions and normalized EMG data for experimental
231 conditions in sitting and standing positions respectively (hypothesis 1). And the comparison of
232 normalized EMG data with respect to the default sitting and standing positions (hypothesis 2) alpha
233 value was set at $p < 0.05$. The differences between each of the gait imagery conditions in the sitting or
234 standing position (hypothesis 3) were explored with Friedman tests with post-hoc Wilcoxon tests. As
235 normalizaed data for three experimental imagery conditions were compared and the alpha value was
236 calculated using Bonferroni's adjustment as $0.05/3$ and set at $p < 0.017$). In addition the effect size for
237 non-parametric data (Fritz, Morris, & Richler, 2012) Z values were computed. All statistical analysis
238 were performed using Statistica 9.0.

239

240 Results

241 For all tested muscles in rest default sitting and standing position the EMG activity was almost
242 silent, the mean and standard deviation reference rest electromyography data [μ V] are presented in
243 **Table 1**. All experimental gait imagery conditions were normalized as a percentage of the rest values
244 separately for each posture, muscle and participant, descriptive statistics of these data are presented
245 in **Table 2**. First gait imagery task in standing position had facilitatory effect on proximal lower limb
246 muscle activity (Table 2, Table 3). However, EMG activity of distal leg muscles decreased for all gait
247 imagery tasks in the sitting position, when the proprioceptive feedback was less appropriate.

248

249 TABLE 1

250 Table 1 insert here

251 TABLE 2

252 Table 2 insert here

253

254 *Gait imagery tasks vs. rest (Hypothesis 1)*

255 Conditions using rhythmic gait imagery mostly indicated an inhibitory effect on lower limb
256 muscle activity compared to the rest default positions (**Table 3**). In the sitting position this was
257 apparent for GM and GL and for TA in all experimental conditions, for BF and ST during gait imagery
258 and simultaneous gait observation and gait imagery after gait execution.

259 In the standing position significant inhibition was only present in GL for second gait imagery
260 condition and in TA for second and third gait imagery condition. In the standing position, the first
261 gait imagery task in the proximal tested muscles (BF, RF) resulted in an increased EMG activity.

262 TABLE 3

263 Table 3 insert here

264

265 *Standing vs. sitting position (Hypothesis 2)*

266 When comparing of the normalized EMG data between experimental conditions and between
267 the sitting and standing positions, muscle activity was mostly higher in the standing position (**Table**
268 **3**). This support the hypothesis that standing facilitates muscle activity in comparison to sitting. The
269 difference were significant for GL ($p < 0.01$, $ES > 0.3$) and BF ($p < 0.05$, $ES > 0.3$) in every experimental
270 condition, for GM and TA ($p < 0.05$, $ES > 0.3$) in the first (SI1 \times TI1) and third gait imagery condition
271 (SI3 \times TI3), for ST and RF ($p < 0.05$, $ES > 0.3$) in first gait imagery (SI1 \times TI1) and imagery during
272 gait observation (SI2 \times TI2) conditions.

273

274 *Subsequent gait imagery tasks (Hypothesis 3)*

275 When comparing experimental conditions, in sitting position the EMG activity was lower
276 during the rhythmic gait imagery after rhythmic gait execution in comparison to the second gait
277 imagery condition for GM ($Z=2.83$, $p=0.005$, $ES=0.36$), GL ($Z=3.24$, $p=0.001$, $ES=0.038$), and TA
278 ($Z=3.73$, $p<0.001$, $r=0.49$) and in comparison to the first gait imagery condition (SI1 \times SI3) for GM
279 ($Z=2.64$, $p=0.01$, $ES=0.39$), GL ($Z=2.79$, $p<0.001$, $ES=0.44$), and TA ($Z=3.63$, $p<0.001$, $ES=0.51$).
280 In the standing position, the muscle activity was lower in the third tested condition compared to the
281 first tested condition for RF ($Z=3.05$, $p<0.001$, $ES=0.42$). For other comparisons the values did not
282 differ significantly.

283

284 *Discussion*

285 Guillot (2007) showed that MI was accompanied by subliminal EMG activity of muscles
286 participating on imagined movement execution. However the increase of lower limb muscle activity
287 during rhythmic gait imagery was not major finding in our study. Lower limb muscles mostly
288 decreased EMG activity during the experimental tasks using gait imagery compared to the rest
289 conditions, where EMG activity of all muscles was almost silent (Table 1). This was significant
290 especially for distal leg muscles in the sitting position (Table 2 and Table 3). The muscle activity
291 increase during MI compared to rest conditions was previously demonstrated mostly for upper limb
292 tasks (Bakker, *et al.*, 1996; Guillot, Di Rienzo, *et al.*, 2012; Guillot, *et al.*, 2007; Solodkin, *et al.*,
293 2004) or for non-gait foot tasks (Bakker, *et al.*, 2007; Bonnet, *et al.*, 1997). To follow on from the
294 results of Bakker *et al.* (2008) it could be suggested that during gait imagery compared to imagery of
295 non-gait or postural foot task supraspinal control is suppressed to some extent. Bakker *et al.* (2008)
296 compared corticospinal excitability within motor imagery of simple foot task (dorsiflexion) and MI
297 of gait measured by motor evoked potentials from task-related muscle m. tibialis anterior in sitting
298 position. They found that motor evoked potentials areas increased during motor imagery of simple
299 foot task, however corticospinal excitability within gait imagery increased just in selected group of
300 subjects (5 from 16) who had larger increase during imagined foot dorsiflexion, so compared to the
301 majority of participants this simple task did not show an increase in muscle activity during gait
302 imagery.

303 As supraspinal control might be suppressed during imagery of postural task we speculate that
304 the less expressed effect of gait imagery on muscle activity could be influenced by neural gait control
305 mechanisms. Rhythmic complex patterns of synergistic muscle activity required for locomotion are
306 to great extent under control of neural autonomy of CPG, neural networks located in lumbosacral
307 spine connected with supraspinal motor regions and with lower limb afferent peripheral sensors
308 (Solopova *et al.*, 2015, Dietz, 2003, 2010; Chvatal & Ting, 2012; Dietz, 2003; MacKay-Lyons, 2002).

309 Motor imagery of lower-limb movements including gait relies mainly on the supplementary motor
310 area, cerebellum, putamen, and parietal regions (Hetu et al., 2013). Activity of these areas is required
311 more for gait planning with respect to changes of external environment rather than for stereotype
312 locomotion which has been shown to be more automatic (Hetu, *et al.*, 2013; la Fougere, *et al.*, 2010).
313 Activity of CPG might be modulated to a great extent by afferent sensory feedback from lower limb
314 receptors even with suppressed supraspinal control than has been previously demonstrated on spinal-
315 cord-injured humans (Bussel et al, 1996, Dietz, 2003, 2010; Harkema, *et al.*, 1997;) or in situations
316 without any extra demands on gait with respect to e.g. additional task or changes in the external
317 environment (Bussel, *et al.*, 1996; Calancie, *et al.*, 1994). Particularly phasic peripheral sensory
318 information associated with lower limb loading during walking evokes lower limb muscle activity
319 (Harkema et al., 1997). Harkema et al. (1997) found that by 70% unloaded body weight stepping (but
320 not 100% unloaded body weight stepping) movements induced by a driven gait orthosis on a treadmill
321 in healthy subjects elicited muscle activity of distal extensor lower limb muscles, namely
322 gastrocnemius medialis and soleus. So the EMG activity of distal lower limb muscles during the gait
323 is to a great extent dependent on phasic peripheral sensory information especially in situations when
324 no extra attention or demands on posture control are needed. The importance of proprioceptive
325 feedback for muscle activity during walking was suggested further McCrea (2001), who found that
326 feedback from extensor proprioceptors induces locomotor dependent reflexes that contribute
327 considerably to extensor muscle activity during real walking. So it is probable that especially distal
328 lower limb motor neurons don't receive enough facilitatory inputs to evoke muscle activity during
329 stereotype rhythmic gait imagery tasks in sitting position. Furthermore it seems that during ~~the~~
330 imagining of gait in a position in which walking is impossible dominate inhibitory effect over possible
331 facilitatory on the muscle activity.

332 The emerging question from these current findings is not only why tested gait imagery
333 conditions do not have facilitatory effect on muscle activity, which was the major focus in previous
334 studies, but why gait imagery tasks resulted in decreased muscle activity compared to the rest
335 condition in our experiment.

336 To date a decrease of EMG activity during imagination of movement execution task has not
337 been described. Decreased excitability of motor neural system during movement imagery compared
338 to rest condition, specifically decreased activity of corticospinal tract, has been previously reported
339 for imagination of muscle relaxation (Kato, Watanabe, Muraoka, & Kanosue, 2015) or during
340 imagination of suppressing movements (Sohn, Dang, & Hallett, 2003) for upper limb tasks. Few
341 studies found decreased corticospinal excitability during imagination of postural tasks in comparison
342 to rest conditions (Hiraoka, 2002; Oishi et al., 1994). Hiraoka (2002) suggested that imagination of
343 stumbling in standing posture lead to decrease excitability of soleus H-reflex and Oishi (1994) found

344 that imaginary of skating motion in elite skate sprinters led to suppression of soleus H-reflex during
345 whole period of imaginary movement. All these finding are support the previous suggestion that
346 motor commands during motor imagery must be inhibited throughout the neural system to some
347 extent to prevent overt movement execution (Guillot, 2007; Jeanarod, 2001) as EMG activity (if
348 present) is just at subliminal intensity without tonic specific activity as during real movement (Guillot,
349 2007; Guillot, 2012; Jeanarod, 2001).

350 Inhibitory processes, which presumably propagate to the spinal motoneurons in parallel with
351 the excitatory inputs might have origin on the cortical, brainstem or either on spinal level (Jeannerod,
352 2006; Prut & Fetz, 1999). We speculate that the cause of EMG decrease, which occurred mostly in
353 sitting position during gait imagery tasks, presumably mostly took place on spinal level as sitting and
354 standing differs mostly by means of different proprioceptive input. It is probable that muscle spindle
355 afferents is gating the strength of Ia afferent synaptic input onto target motor neurons during gait
356 imagery in the same way as during gait execution (MacKay-Lyons, 2002). One of proposed
357 mechanisms of muscle activity inhibition is presynaptic inhibition according to a previous finding
358 that soleus H-reflex excitability as function of EMG level is decreased during gait (Stein & Capaday,
359 1998). Presynaptic inhibition reduces the amount of neurotransmitter released at the presynaptic
360 terminal of the Ia axon which lead to decrease in EMG activity (Brooke et al., 1997; Bonnet et al.
361 1997). Furthermore we speculate that muscle activity decrease during gait imagery task might be
362 influenced by depression of afferent neuronal discharge as has been demonstrated during fictive
363 locomotion in the cat induced by mesencephalic locomotor region stimulation (Perreault et al., 1999).
364 Decrease of muscle and cutaneous afferent-evoked monosynaptic field potentials reflected a
365 reduction of depolarizing synaptic current into spinal neurons during fictive locomotion (Perreault et
366 al., 1999).

367

368 *The influence of posture*

369 For all tested muscles in most of experimental conditions was muscle activity during gait imaginary
370 tasks significantly lower in sitting position compared to muscle activity during gait imaginary tasks
371 in standing position (see Table 2, 3). Thus, the standing position compared to sitting position had an
372 excitatory effect on muscle activity during rhythmic gait imagery tasks. Standing posture is congruent
373 with walking and thus offer more appropriate somatosensory (tactile, proprioceptive and visual)
374 feedback compared to incongruent positions with walking such as sitting or lying. Presence of real
375 somatosensory feedback facilitates activity of neural structures within motor imagery and motor
376 observation (Mizuguchi et al., 2012; Vargas et al., 2004). Mizuguchi et al. (2012) found that
377 imagination of squeezing the ball and holding the real ball at the same time enhanced the MEPs in
378 comparison to the same situation just without the ball. Vargas et al. (2004) observed that corticospinal

379 excitability increased in situation when hand posture was compatible with the imagined task
380 compared to incompatible hand posture with the imagined task. Saimpont et al., 2012) proved that
381 posture might influence even accuracy of imagined movement, in their experiment the time duration
382 of gait motor imagery in standing posture (body posture congruent with walking) was more
383 comparable with real gait than gait motor imagery in sitting posture. It has been also previously
384 shown, that standing posture compared to supine posture (the one most used throughout the studies
385 concerning effect of gait observation or gait imagery) has excitatory effect on neural structures
386 (Nakazawa et al., 2003; Shimba et al., 2010). Nakazawa et al. (2003) demonstrated that both stretch
387 reflex and MEP elicited in tibialis anterior were significantly greater in standing compare to supine
388 posture (background EMG was silent in both conditions). Shimba et al. (2010) found that even passive
389 standing posture (accomplished by using gait orthosis) had higher impact on increased stretch reflex
390 of m. soleus compared to supine position. This might reflect facilitatory effect of standing position
391 on muscle spindle Ia afferent fibers. Facilitation of muscle spindle activity with respect to position
392 congruent with imaginary movement found also Bonnet et al. (1997). In their study they showed that
393 mental simulation of pressure on a pedal with the foot in reclined sitting position with their feet on
394 two pedals led to larger changes in T-reflex amplitude compared to H-reflex amplitude (activity of
395 muscle spindle Ia afferent fibres is elicited within the T-reflex, but not by H-reflex) in the leg involved
396 in the simulation. Even the extension of the hip in the standing position might have facilitatory effect
397 on muscle activity compared to sitting position, because also afferent input from hip joints is
398 important for the leg muscle activation during locomotion in dependence on hip position (Dietz and
399 Duysens, 2000; Dietz et al., 2002; Grillner & Rossignol, 1978). Grillner & Rossignol (1978)
400 previously proved that preventing the hip from extension in chronic spinal cats inhibits the flexors
401 muscle activity. As EMG activity depends on level of motoneuron pool excitation it is probable that
402 muscle proprioceptive (muscle spindle) afferents is gating the strength of Ia afferent synaptic input
403 onto target motoneurons during gait imagery, same as during gait execution (MacKay-Lyons, 2002).
404 Then the level of proprioceptors activation might be crucial for the the subthreshold activation of
405 target muscles during gait imagery tasks. This assumption is in accordance with previous studies the
406 appropriate proprioceptive feedback (concretly posture congruent with imaginary task) provided
407 excitatory input to the motor system and facilitates muscle activity.

408 For the proximal tested muscles (BF and RF) the gait imagery task in the standing position
409 was the only experimental condition when the muscle activity increased compared to the rest position.
410 It has been previously suggested that the proximal leg muscles (e.g., BF) are mostly controlled by the
411 monosynaptic corticospinal pathways compared to mostly polysynaptic corticospinal innervations of
412 the distal leg muscles (e.g., GM) (Brouwer & Ashby, 1991; Cowan, Day, Marsden, & Rothwell,
413 1986). So presumably during the gait imagery task, the direct neural input from the cortex to the

414 motoneuron may enhance the ability of the cortex to control the proximal leg muscles (Brouwer &
415 Ashby, 1991). This assumption is in accordance with previous findings that during hand movements
416 dominates monosynaptic cortical-motoneuronal input (Nicolas et al., 2001) and mostly for upper limb
417 movements the presence of EMG activity during imagery tasks has been already demonstrated. It is
418 possible that motor imagery does not provide equivalent neural input to proximal and distal leg
419 muscles, but this has to be further explored. And still just biarticular lower limb muscles were
420 measured. To follow our results it is likely that the imagining of rhythmic gait provides inhibitory
421 input mostly to the distal leg muscles in the default sitting position. In accordance to previously
422 mentioned studies inhibition might reflect the summation of several factors including: decreased
423 supraspinal effort for stereotype gait imagery tasks, spinal inhibitory mechanisms (presynaptic
424 inhibition), different neural drive to the motoneurons of distal and proximal leg muscles, and default
425 sitting posture which does not provide appropriate feedback for real walking. However the results of
426 this study are limited to young woman population with good imagery ability, and to stereotype
427 rhythmic gait imagery task. Therefore, further research is required with respect to different genders
428 and populations.

429

430 *Comparison of EMG activity during experimental conditions*

431 Combination of motor imagery and observation (Wright, Williams, & Holmes, 2014) or
432 previous imagined movement execution (Wriessnegger, Steyrl, Koschutnig, & Muller-Putz, 2014)
433 enhances activity of neural structures and motor learning processes (Gomes, *et al.*, 2014; Nedelko,
434 Hassa, Hamzei, Schoenfeld, & Dettmers, 2012) compared to motor imagery itself. Based on this
435 assumption we hypothesized, that both simultaneous motor imagery with motor observation and
436 previous execution of imagined movement would have further facilitatory effect on muscle activity
437 compared to gait imagery alone. So we added these “augmented” imagery conditions in given order
438 to the experimental protocol. However in our experiment the second and the third experimental
439 condition mostly led to muscle activity decrease compared to the first tested situation. As the order
440 of first, second and third experimental conditions were not randomized we suggest that the decrease
441 in muscle activity within repeated tested motor imagery tasks in our experiment might reflect to some
442 extent the gradual habituation effect. It has been previously described, that cortical activity is mostly
443 pronounced during initial trials of complex motor imagery tasks (imagery of volleyball spike attack)
444 compared to second and third motor imagery where the short-term habituation effect might be present
445 (Stecklow et al., 2010). None of tested participants reported feelings of tiredness during the
446 experiment the mental fatigue, which has been previously reported for prolonged imagery tasks
447 (Rozand et al., 2016), was not the reason of decreased muscle activity for subsequent imagery tasks.

448 We suggest here that more challenging imagery tasks as part of gait rehabilitation are required, then
449 habituation effect might be avoided (Marchal-Crespo et al., 2014).

450

451 The results of this study potentially further our understanding of influence of rhythmic gait
452 imagination on lower limb muscles with respect to the body posture. This in turn provides important
453 information for gait imagery rehabilitation protocols and could increase our understanding of gait
454 control mechanisms.

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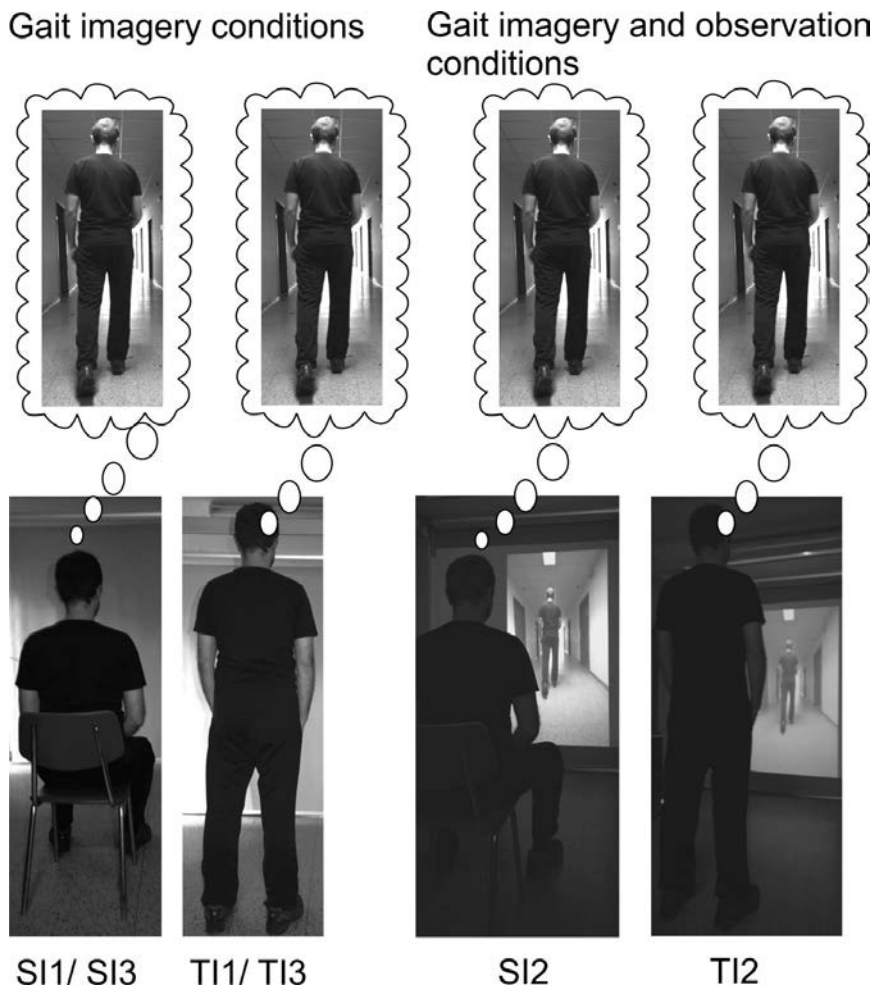
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763 FIGURE 1

764 Illustration of tested experimental conditions



765 SI1/ SI3

765 TI1/ TI3

765 SI2

765 TI2

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767 TABLE 1

768 Mean EMG [μ V] reference values (\pm SD) for all tested muscles in default sitting and standing positions

	Gastrocnemius medialis		Gastrocnemius lateralis		Tibialis anterior		Biceps femoris		Semitendinosus		Rectus femoris	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Sitting position	1.35	0.53	1.48	0.52	1.59	0.53	1.3	0.39	1.2	0.4	1.21	0.48
Standing position	6.17	3.72	3.65	1.79	2.45	0.96	2.6	2.57	2.82	3.6	1.72	1.4

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771 TABLE 2

772 Normalized elektromyographic activity with respect to reference value for every muscle [%] during
 773 gait imagery tasks in sitting and standing position

		Gait imagery		Gait imagery and observation		Gait imagery after gait execution	
		<i>Med</i>	<i>IQR(Q1 – Q3)</i>	<i>Med</i>	<i>IQR (Q1 – Q3)</i>	<i>Med</i>	<i>IQR (Q1 – Q3)</i>
Gastrocnemius lateralis	S	73.15	(58.31–97.48)	69.07	(54.05–92.82)	61.62	(45.73–84.55)
	T	95.33	(85.23–127.63)	87.31	(70.27–95.68)	89.85	(81.15–106.95)
Gastrocnemius medialis	S	80.64	(54.15–97.92)	79.13	(51.56–98.24)	60.22	(45.91–91.24)
	T	97.19	(78.13–129.47)	84.53	(70.58–109.11)	91.09	(75.83–122.77)
Tibialis anterior	S	75.24	(64.25–112.14)	77.7	(62.84–95)	59.53	(50.49–86.9)
	T	96.58	(75.73–119.36)	88.13	(82.11–99.03)	85.34	(70.78–103.78)
Biceps femoris	S	117.9	(91.09–221.63)	101.49	(86.37–151.14)	104.77	(82.31–129.04)
	T	93.5	(88.57–103.43)	91.48	(82.49–102.03)	85.86	(78.97–98.64)
Semitendinosus	S	92.26	(78.35–108.78)	88.40	(76.73–102.7)	87.33	(76.62–107.02)
	T	111.28	(89.03–158.43)	99.1	(87.29–129.14)	98.15	(71.07–148.37)
Rectus femoris	S	91.32	(86.17–106.95)	90.33	(82.06–100.34)	90.83	(75.08–104.5)
	T	111.11	(93.8–270.79)	98.3	(84.09–156.77)	97.24	(78.19–154.44)

S – sitting position, T – standing position, Med – median, (Q1 – Q3) – (25th – 75th percentile of data)

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782 TABLE 3

783 Results of statistical analysis (Wilcoxon signed rank test and Effect Size) of changes in the muscle
 784 activity during gait imagery tasks

		gait imagery tasks in the sitting position compare to default sitting rest position			gait imagery tasks in the standing position compare to default standing rest position			gait imagery tasks in the sitting position compare to gait imagery tasks in the standing position		
		<i>Wilcoxon's</i>		<i>Effect</i>	<i>Wilcoxon's</i>		<i>Effect</i>	<i>Wilcoxon's</i>		<i>Effect</i>
		<i>Z</i>	<i>P</i>	<i>Size</i>	<i>Z</i>	<i>P</i>	<i>Size</i>	<i>Z</i>	<i>P</i>	<i>Size</i>
Gastrocnemius medialis	I1	3.00	<.001	0.41	0.29	0.77	0.04	2.21	0.03	0.3
	I2	3.15	<.001	0.43	1.78	0.08	0.24	1.42	0.16	0.19
	I3	4.08	<.001	0.56	0.29	0.77	0.04	3.99	<.001	0.54
Gastrocnemius lateralis	I1	3.29	<.001	0.45	0.65	0.52	-0.08	4.30	<.001	0.58
	I2	4.04	<.001	0.55	2.71	0.01	0.34	2.79	0.01	0.38
	I3	4.42	<.001	0.6	1.15	0.25	0.16	4.18	<.001	0.59
Tibialis anterior	I1	2.16	0.03	0.29	0.36	0.72	0.05	2.38	0.02	0.32
	I2	2.26	0.02	0.31	2.07	0.04	0.28	1.39	0.16	0.19
	I3	3.89	<.001	0.53	2.81	<.001	0.38	3.08	<.001	0.42
Biceps Femoris	I1	1.71	0.09	0.23	2.16	0.03	-0.29	2.59	0.01	0.35
	I2	3.05	<.001	0.42	1.13	0.26	-0.15	2.64	0.01	0.36
	I3	3.10	<.001	0.42	0.77	0.44	-0.11	1.99	0.05	0.271
Semitendinosus	I1	1.42	0.16	0.19	1.75	0.08	0.24	2.50	0.01	0.34
	I2	3.17	<.001	0.43	0.53	0.60	-0.07	1.80	0.07	0.26
	I3	2.09	0.04	0.28	0.22	0.83	-0.03	1,13	0.26	0.15
Rectus femoris	I1	1.18	0.24	0.16	2.45	0.01	-0.33	3.39	<.001	0.46
	I2	1.95	0.05	0.27	0.86	0.39	-0.12	2.35	0.02	0.32
	I3	1.49	0.14	0.2	0.26	0.79	-0.04	0.96	0.34	0.13

I1 - gait imagery, I2 - gait imagery and observation, I3 - gait imagery after gait execution

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