

# 1           **The effects of different frequencies of rhythmic acoustic stimulation on gait** 2                           **kinematics and trunk sway in healthy elderly population**

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16   **Keys words: 3D gait analysis, kinematics, stability, cadence, trunk displacement index, falls**

## 17 18   **ABSTRACT**

19       The use of rhythmic acoustic stimulation (RAS) in improving gait and balance in healthy elderly subjects  
20   has been widely investigated. However, methodologies and results are often controversial. In this study, we  
21   hypothesize that both the kinematic features of gait and stability, depend on the frequency at which RAS is  
22   administered. Our aim was to observe, through 3D Gait Analysis, the effect of different types of RAS (at a  
23   fixed frequency or based on the average cadence of each subject) on both gait spatio-temporal parameters  
24   and stability. The latter was estimated through an innovative measure, the trunk displacement index (TDI)  
25   that we have recently implemented. We observed that the low frequencies RAS led to a general slowdown  
26   of gait, which did not provide any clear benefit and produced harmful effects on stability when the frequency  
27   became too low compared to the individual natural frequency. On the contrary, the high frequencies of RAS  
28   showed a slight acceleration of gait, accompanied by better stability (as documented by a lower TDI value),  
29   regardless of the type of RAS. Finally, the RAS equal to the individual natural cadence also produced an  
30   increase in stability.

## 31 **1. INTRODUCTION**

32 Aging-related motor impairment arises from multiple factors, including bone loss, muscle  
33 atrophy, and a decline of both central and peripheral nervous systems functioning<sup>1</sup>. As a  
34 consequence, the elderly people show a reduction of the static and dynamic balance, slower  
35 movements, altered modulation of strength and increased walking variability<sup>2-4</sup>. All these factors  
36 are closely related and contribute to increased instability and risk of falls<sup>5</sup>. Due to the frailty of  
37 elderly people, falling carries a high burden, as it compromises personal autonomy, limits mobility  
38 and increases morbidity and mortality. Indeed, several studies highlighted the close connection and  
39 the increasing rate of death originating from fall events, addressing it as an important public health  
40 problem<sup>6-8</sup>. Additionally, in the elder population, cognitive decline and some psychological factors,  
41 such as stress, anxiety and depression contribute to an increased risk of falling and a worsened  
42 quality of life<sup>9</sup>. Hence, finding preventive strategies is needed.

43 In order to quantify instability, identify weak points and try to minimize these risks, several  
44 studies have investigated movement and, in particular, locomotion in elderly subjects, using  
45 different methodologies and tools<sup>10-12</sup>. Among these, the 3D gait analysis (3D-GA), typically  
46 considered the gold standard<sup>31</sup>, is a quantitative method to measure the kinematics of movements,  
47 widely applied for the assessment of motor skills, in physiological and pathological conditions<sup>13-19</sup>.

48 Many studies have investigated the strategies adopted to counterbalance the increased (static  
49 and dynamic) instability, in physiological or pathological conditions. Among these, several works  
50 focused on the effects of sensory stimulation on balance and locomotion<sup>20-23</sup>. Sensory afferents  
51 provide information on the reciprocal position and movement of body segments and their  
52 orientation in space<sup>20</sup>. The increase in sensory information (auditory, visual or vibro-tactile  
53 stimulations) can help static and dynamic balance, even for individuals with sensory and motor  
54 impairment<sup>24</sup>. Accordingly, it has been widely demonstrated that an appropriate acoustic stimulus  
55 improves walking parameters in different pathological conditions, including Parkinson's disease<sup>25-</sup>  
56 <sup>27</sup>, stroke and multiple sclerosis<sup>28</sup>.

57 Given the therapeutic potential, some effort has been devoted to the description of the  
58 mechanisms through which acoustic stimuli, and rhythmic acoustic stimulation (RAS) in particular,  
59 influence walking. This is particularly relevant for the possibility of using a device based on RAS to  
60 support motor rehabilitation even in elderly people with a high risk of falls. Dickstein et al. studied  
61 how walking synchronizes to an acoustic stimulus emitted by a metronome, at different fixed (i.e.

62 irrespective of each participant's baseline frequency) frequencies (60, 110, and 150 beats/min). The  
63 authors showed that lower frequencies display greater ease of synchronization<sup>29</sup>. Conversely, Yu et  
64 al. explored the effects of three different frequencies based on the cadence of each participant (i.e.  
65 90%, 100% and 110% of the average cadence of each participant), on spatiotemporal gait  
66 parameters<sup>30</sup>. It was suggested that the faster stimulus affects the most gait parameters, such as  
67 the stride length, the cadence and the walking speed, as compared to the non-cued walking. Hence,  
68 how to most effectively set the stimulus features remains an open question, as it is not clear which  
69 gait parameters and cortical processes are involved in the adaptation to acoustic stimulation.

70 Most of the studies have focused selectively on the lower limbs, excluding the other body  
71 segments<sup>14</sup>. However, in order to maintain balance<sup>32</sup>, walking entails through synergistic and  
72 coordinated movements of the upper limbs, trunk and lower limbs<sup>33</sup>. Importantly, central balance  
73 control does not intervene on each muscle individually, but rather aims at the control of the centre  
74 of mass (COM), with segmental or sub-segmental adjustments left to hierarchically lower  
75 mechanisms<sup>32,34,35</sup>. Therefore, studying the static and dynamic balance of a population with higher  
76 risk of falling, such as the elderly, through the exclusive study of the lower limbs, is highly limiting.

77 We have recently implemented an innovative measure of postural stability which assesses the  
78 trunk displacement in relation to the centre of mass, that we named trunk displacement index (TDI).  
79 This measure, obtained from 3D-GA data, evaluates the ratio of the displacements of trunk and COM  
80 on three anatomical planes, conveying the overall motor performance. Considering that a less  
81 efficient control of the COM is related to increased risk of falling, we hypothesize that the  
82 effectiveness of different acoustic stimuli on the gait stability can be evaluated assessing the TDI  
83 value.

84 The aim of this work is to evaluate the effect of different frequencies (fixed or established on  
85 the average cadence of each subject) of RAS on walking in healthy elderly subjects. As shown,  
86 studies on the effect of RAS on gait used a variety of methods producing conflicting results. We  
87 hypothesized that optimal gait stabilization does not occur at the same frequency for each subject,  
88 rather, the optimal frequency of stimulation is subject-specific and span around the individual  
89 cadence of each subject. In order to test our hypothesis, we used 3D-GA to calculate spatio-  
90 temporal gait parameters and TDI in three experimental conditions characterized by a RAS  
91 frequency lower, equal or higher than that of each subject<sup>34</sup>. More precisely, each subject was  
92 recorded while walking at a RAS frequency which was: 1) equal to his/her cadence, 2) lower (90%

93 of the basal cadence and 80 bpm) and 3) higher (110% of the basal cadence and 120 bpm).  
94 Furthermore, we carried out the correlation analysis in order to evaluate the relationship between  
95 the gait parameters and stability, expressed as TDI.

96

## 97 **2. METHODS**

### 98 **2.1 Participants**

99 Twenty-two elderly people were recruited (table 1), nine of which were excluded because they  
100 did not meet the following inclusion criteria: (i) 65 to 85 years old; (ii) no skeletal, muscular and  
101 neurodegenerative disorders; (iii) no hearing impairment; (iv) Beck Depression Inventory (BDI)<sup>36</sup>  
102 score < 13; (v) Mini Mental State Examination (MMSE) score > 23,80<sup>37</sup>; (vi) Frontal Assessment  
103 Battery (FAB) score > 12.03<sup>38</sup>. Thirteen participants (6 males and 7 females) were included. Written  
104 informed consent was obtained from all participants prior to participation, in accordance with the  
105 declaration of Helsinki. The study was approved by the local Ethic Committee (University of Naples  
106 Federico II, n. 26/2020).

### 107 **2.2 Gait Analysis Assessment**

#### 108 *2.2.1 Protocol*

109 The 3D-GA was carried out in the Motion Analysis Laboratory of the University of Naples  
110 “Parthenope”. The 3D-GA data were acquired through a Stereophotogrammetric system, equipped  
111 with eight infrared cameras (ProReflex Unit-Qualisys Inc., Gothenburg, Sweden). In agreement with  
112 the modified Davis protocol<sup>39</sup>, fifty-five passive markers were positioned on each participant on  
113 anatomical landmarks of the feet, the lower limb joints, the pelvis and the trunk, as well as on the  
114 upper limb joints and on the head (Fig.1). The recorded data were processed using a tracking data  
115 software (Qualisys Track Manager by Qualisys AB, Göteborg, Sweden) and a software (Visual 3D by  
116 C-Motion Inc., Germantown, MD) to rebuild a model of the skeleton<sup>16,40</sup>. We recorded four trials for  
117 each frequency and each trial included four consecutive steps (a gait cycle), similarly to Liparoti et  
118 al.,<sup>15</sup>. In order to obtain a more reliable estimate, we calculated the average of each step within a  
119 trial. Participants were asked to walk at the pace of the acoustic stimulus, emitted by a metronome  
120 (MA-1 Solo Metronome, Korg - UK). The acoustic stimulation was set to have a tone of 440 Hz<sup>41</sup>(such  
121 as a metronome tic), with an easily audible volume<sup>42</sup>.

122 The data acquisition was carried out in two times. During the first one, subjects were recorded  
123 at a self-selected cadence in order to calculate the natural cadence of each participant. In the second  
124 phase, we recorded the participants' walking in 6 experimental conditions: 1) simple walking (SW);  
125 2) walking with RAS at frequency equal to the subject-specific average cadence (AC) (100% AC); 3)  
126 walking with RAS at a frequency equal to 90% of the participants' cadence (90% AC); 4) walking with  
127 RAS at a frequency equal to the 110% of the participants' cadence (110% AC); 5) walking with RAS  
128 at fixed frequency equal to 80 beat per minute (bpm); 6) walking with RAS at fixed frequency of 120  
129 bpm (table 2). The order of the acoustic stimuli was randomized to reduce the learning effect and  
130 fatigue. For the SW, all participants were instructed to walk at a normal pace over a 10-meter-long  
131 carpet.

### 132 2.2.2 Spatio-temporal Parameters

133 3D-GA has been used to obtain the temporal and spatial parameters of gait. The former included  
134 Speed, Stance Time, Swing Time, Cycle time and Double Limb Support time (DLS). The latter included  
135 Stride Width and Stride Length. The variability coefficient (CV) (the ratio between standard deviation  
136 and average value x 100 (%)) was calculated for all spatio-temporal parameters, except speed.

### 137 2.2.3 Trunk Displacement Index

138 In order to evaluate the stability, we calculated the TDI<sup>34</sup>, a newly described synthetic index,. We  
139 measured the trajectory of the centre of mass ( $COMt$ ) in the three-dimensional space, during gait  
140 and calculated its mean ( $\overline{COMt}$ ). Thereafter, we calculated the same three-dimensional trajectory  
141 for the upper trunk ( $Tt$ ). Subsequently, we calculated separately the distances between  $COMt$  and  
142  $Tt$  from  $\overline{COMt}$  in each registered frame of the gait cycle of the individual, obtaining a vector of  
143 distances ( $COMd$  and  $Td$  respectively) for each one of the three planes, as shown in equations (1  
144 and 2):

$$145 \quad COMd = COMt - \overline{COMt} \quad (1)$$

$$146 \quad Td = Tt - \overline{COMt} \quad (2)$$

147 Finally, to enclose all the information in a unique parameter, which conveys the three-  
148 dimensional displacement of the trunk, we performed the following two final steps. Firstly, we  
149 summed the norm of each vector of distances ( $COMd$  and  $Td$ ). Then, we calculated the ratio  
150 between those two values obtaining a unique dimensionless number, as shown in the equation (3),

151 representative of the relationship between the three-dimensional displacement of the trunk and  
152 the COM<sup>34</sup>.

$$153 \quad TDI = \frac{\sum \|Td\|}{\sum \|COMd\|} \quad (3)$$

## 154 **2.3 Statistical analysis**

155 The statistical analysis was performed in Matlab (Mathworks®, version R2018b). The means and  
156 standard deviations of the gait parameters were calculated after correcting each variable by the  
157 body mass index (BMI) of each subject. Gait parameters were compared among all conditions,  
158 analysing three subsets separately: the first subset included the SW group and the high frequency  
159 stimuli groups (110% AC e 120 bpm); the second subset included the SW group and the low  
160 frequency stimuli groups (90% e 80 bpm); the third subset included the SW and the 100% AC groups.

161 The Shapiro-Wilk test was used to check the normal distribution of variables. Given the  
162 heterogeneous distribution of the variables and the small size of the sample, we performed  
163 nonparametric statistical testing. Friedman test was used to investigate the differences within  
164 subgroups, while Wilcoxon signed rank test was used to perform the pairwise comparison.  
165 Spearman correlation tests were carried out to test the relationship between TDI and gait  
166 parameters including all conditions.

167

## 168 **3. RESULTS**

### 169 **3.1 Simple Walking – 100% AC frequency**

170 Regarding the Temporal Parameters, there was a statistically significant difference in the DLS  
171 time ( $p < 0.01$ ) between SW and 100% AC, with the latter showing higher values. About the Spatial  
172 Parameters, at 100% AC it was observed increased Stride Length ( $p < 0.05$ ), as well as reduced DLS  
173 CV ( $p < 0.01$ ), as compared to SW. Moreover, at the 100% AC the TDI was decreased as compared  
174 to SW ( $p < 0.01$ ) (Fig. 2).

### 175 **3.2 Low Frequencies**

#### 176 *3.2.1 Simple Walking - 90%AC Frequency*

177 The comparison between SW and the 90% AC frequency showed differences in the Temporal  
178 Parameters. In particular, the reduced frequency implied a statistically significant decrease of Speed  
179 ( $p < 0.05$ ) and an increase of Stance Time ( $p < 0.001$ ), Cycle time ( $p < 0.001$ ) and DLS time ( $p < 0.001$ ).

180 However, no significant difference was found in Spatial Parameters and in the TDI value in the  
181 comparison between these two conditions (Fig. 3).

### 182 *3.2.2 Simple Walking - 80 bpm Frequency*

183 With regard to the Temporal Parameters, the walking at fixed frequency set at 80 bpm caused a  
184 statistically significant reduction of the Speed ( $p < 0.001$ ), and an increase of the Stance time ( $p <$   
185  $0.001$ ), Swing time ( $p < 0.01$ ), Cycle time ( $p < 0.001$ ) and DLS time ( $p < 0.001$ ) as compared to SW.  
186 For the Spatial Parameters, it was shown a decrease of the Stride Length ( $p < 0.05$ ). Furthermore,  
187 this frequency of stimulation also caused increased Stance time CV ( $p < 0.05$ ) and Swing time CV ( $p$   
188  $< 0.01$ ). No significant difference was found in the TDI between these two conditions (Fig. 3).

## 189 **3.3 High Frequencies**

### 190 *3.3.1 Simple Walking - 110%AC Frequency*

191 The results showed that the 110% AC frequency affected all Temporal Parameters. In particular,  
192 it was documented statistically significant increase of Speed ( $p < 0.001$ ) and decrease of Stance time  
193 ( $p < 0.05$ ), Swing Time ( $p < 0.001$ ) and Cycle time ( $p < 0.001$ ), compared to the SW. Concerning the  
194 Spatial Parameters, this frequency of stimulation caused a rise of Stride Length ( $p < 0.05$ ). Moreover,  
195 the results showed a reduction of the TDI compared to SW ( $p < 0.01$ ) (Fig. 4).

### 196 *3.3.2 Simple Walking - 120 bpm Frequency*

197 The comparison between the SW and walking at the RAS at 120 bpm showed results resembling  
198 that at the 110% AC frequency. In fact, the results highlighted a rise of the Speed ( $p < 0.001$ ), and a  
199 reduction of the Stance time ( $p < 0.01$ ), Swing time and Cycle time ( $p < 0.001$ ) with the RAS at 120  
200 bpm. Moreover, our data showed increased Stride length ( $p < 0.05$ ) and reduced TDI ( $p < 0.05$ ) (Fig.  
201 4).

## 202 **3.4 Correlations**

203 We performed a correlation analysis to explore the relationship between the TDI and the gait  
204 parameters. The TDI correlated negatively with Speed ( $r = -0.69$ ,  $p < 0.001$ ) and Stride Length ( $r = -$   
205  $0.56$ ,  $p < 0.001$ ), and positively with Stance Time ( $r = 0.32$ ,  $p = 0.012$ ), Swing Time CV ( $r = 0.27$ ,  $p =$   
206  $0.033$ ), Cycle Time ( $r = 0.32$ ,  $p = 0.013$ ), and DLS time ( $r = 0.39$ ,  $p = 0.002$ ) (Figure 5).

207



## 208 **4. DISCUSSION**

209 In this study, we assessed how different frequencies of the RAS may affect walking in healthy  
210 elderly subjects, evaluating the spatio-temporal parameters, and the TDI.

### 211 **4.1 Spatio-Temporal Parameters**

#### 212 *4.1.1 SW and 100% AC*

213 As expected, with regard to the comparison between SW and 100% AC, the results highlighted  
214 that, by setting the frequency of the RAS equal to the cadence of each subjects, the temporal  
215 parameters (speed, stance time, swing time and cycle time) showed no significant change, except  
216 for the DLS time, whereas differences in the spatial parameters (Stride length and DLS CV) were  
217 observed. In fact, while the results of the temporal parameters showed a synchronization with the  
218 RAS, the spatial parameters demonstrated an increase in stability, as suggested by the increase of  
219 Stride length and the reduction DLS CV. Data also revealed a raise of DLS time, commonly related to  
220 altered stability and increased risk of fall<sup>44-46</sup>. We assume that the DLS time increase is due to a  
221 mechanism of synchronization with the stimulus, which involves a longer expectation of the  
222 stimulus with both feet in contact with the ground. To overcome this limitation, we calculated the  
223 TDI, a measure capturing gait stability. Taking into consideration the trunk displacement in relation  
224 to the COM of the individual, the TDI provides a proxy of stability of the whole-body. Indeed, during  
225 the 100% AC frequency stimulation, the TDI value decreased, showing a reduction of trunk swings,  
226 and therefore a greater stability. This agrees with a study performed by Arias et al. on the effects of  
227 rhythmic sensory stimulation on gait in Parkinsonians patients and age-matched healthy controls.  
228 Furthermore, Arias et al. also reported increased speed and reduced stride time variability in the  
229 same subjects<sup>47</sup>. This partial discrepancy could be caused by the difference in age of the population.  
230 Indeed, our participants synchronised with the stimulus and increased the DLS time, rather than the  
231 speed, obtaining a reduction of the DLS CV.

#### 232 *4.1.2 Low Frequencies*

233 The results of the comparison between SW and low frequencies stimulation (90% AC and 80  
234 bpm) showed the same trend for almost all parameters, with the main variations in the temporal  
235 parameters. In fact, for both stimulations, a reduction of Speed, and a rise of Stance time, Cycle  
236 Time and DLS time was observed. In fact, similarly of the 100% AC frequency, even in the low  
237 frequencies the increase of the DLS time could be caused by the same mechanism of synchronization



238 with the stimulus. We speculated that, in order to adapt to the low frequency stimulus, the  
239 participants increased the whole cycle time by increasing the time spent in double stance, in order  
240 to wait for the next stimulus in a condition of higher stability (both feet on the ground, instead of  
241 one, as in the swing phase). The gait modification induced by the RAS at 90% frequency in our  
242 sample are in agreement with a study performed by Willems et al., which found reduced speed and  
243 cadence and unmodified stride length<sup>48</sup>. When reducing the frequency of the stimulus, it can be  
244 observed that all the temporal parameters slowed down. On top of that, the lowest frequency (80  
245 bpm) also showed increased Swing time and rise of Stance Time CV and Swing Time CV. To help  
246 interpretation, the TDI variations of the two stimulations showed opposite trends. In fact, during  
247 the 90% AC frequency stimulation (i.e. the one that was more similar to the natural cadence) we  
248 observed a reduction of the TDI value, and hence greater stability. This shows that the differences  
249 in the spatio-temporal parameters induced by the stimulus, reduced oscillations and results in  
250 enhanced stability, by forcing the subject to walk slightly slower. However, during the 80-bpm  
251 frequency stimulation the stability becomes sub-optimal (i.e. forcing the subject to walk at a much  
252 lower cadence as shown in table 2. Intuitively, this phenomenon could be thought as similar to the  
253 common experience of riding a bicycle: a slow pace makes the riding easier, until the point when  
254 the lack of speed makes oscillations more pronounced, and stability harder to maintain. In other  
255 words, the modification of the spatio-temporal parameters succeeds at maintaining optimal  
256 balance, but fail when the difference becomes too big. This has implication when designing balance-  
257 enhancing strategies such as with physiotherapy.

#### 258 *4.1.3 High Frequencies*

259 Also, in this case, the results of the comparison between SW and high frequencies (110% AC and  
260 120 bpm) showed the same trend for all the parameters. In fact, for the Spatial Parameters, one can  
261 observe longer Stride Length and, for Temporal Parameters, increased Speed, with the consequent  
262 reduction of the Stance Time, the Swing Time and the Cycle Time. Our findings are in accordance  
263 with Yu et al., which found increased Speed, cadence and Stride Length in healthy young subjects<sup>30</sup>.  
264 It can be observed that the high frequencies do not cause alterations in the double support time.  
265 Moreover, both frequencies caused lower TDI (hence smaller oscillations of the trunk over the  
266 COM). Therefore, the gait changes that occur under the higher RAS suggest a better ability to control  
267 the body sway and consequently less body instability.

#### 268 **4.3 Correlations**

269 Our analysis showed that the TDI correlated positively with the DLS time. This finding implies  
270 that, when the TDI increases, the DLS increases too. This seems logical as double stance time is the  
271 most stable part of the gait cycle<sup>49</sup>, and it typically becomes longer when more stability is requested.  
272 Hence, when instability is present, as indicated by higher TDI, the gait is modified toward higher  
273 double support time. In fact, the relationship between the TDI and the DLS time is not the same in  
274 all conditions. While all the three conditions (100% AC, 90% AC, 80 bpm) saw an increase in DLS  
275 time, only the 80 bpm condition showed increased TDI, while the opposite happened in the 100%  
276 AC and 90% AC conditions. Our interpretation of this finding is that the subjects successfully  
277 responded to the 100% AC RAS and the 90% AC RAS by adapting the DLS phase, in such a way as to  
278 obtain more stability, perhaps taking advantage of the external cueing and, consequently,  
279 decreasing the oscillations of the trunk. However, the 80 bpm RAS is much lower than the natural  
280 walking rhythm of our population, and the over-extended duration of the DLS provoked a deficit in  
281 stability, resulting in higher TDI values. In other words, the TDI grows linearly with the DLS for a  
282 certain range of stimulation. However, out of this range, when the stimulus becomes too different  
283 from the natural frequency, such relationship no longer holds, and higher DLS starts to correspond  
284 to lower stability, perhaps as if the compensatory mechanisms can no longer achieve stability. In  
285 the 110% AC and 120 bpm cases, we did not observe any significant change in the DLS time, while  
286 the TDI decreased significantly. This information suggests that the rise of the TDI might be  
287 interpreted as an indicator of a condition of instability.

288 Moreover, the TDI resulted to correlate negatively with the Speed. Consequently, we can  
289 observe that Speed values increase when the TDI values decrease. Significant increase and decrease  
290 of Speed were showed during high and low frequency RAS, respectively. As shown by our results,  
291 the participants adjusted the Cycle Time, in order to follow the low or high RAS, respectively. Hence,  
292 the strategy put in place by participants, to reach the correct Cycle Time, entailed modulating the  
293 Speed. Consequently, walking faster caused shorter duration of the Cycle Time. Again, the example  
294 of the bicycle can be of help, given that a slightly higher speed can stabilize oscillations, similarly to  
295 what we observed in this case.

296 The TDI was correlated negatively to the Stride Length too. Similarly, to our previous line of  
297 thinking, also in this case the presence of optimal balance, as signalled by the low TDI, would mean  
298 that it is safer to make longer steps.

299 Our results agree with several studies which reported that the elderly commonly showed a  
300 reduced speed and short stride length, linked to an impaired stability. In a review on balance and  
301 gait in the elderly, Osoba et al., describe the slow speed and the short stride length as a cautious  
302 gait pattern put in place to increase stability<sup>50</sup>. Furthermore, a clinical guide presented by Pirker and  
303 Katzenschlager on gait disorder in elderly people, states that ageing is related to a reduction in step  
304 length and, consequently, in speed, highlighting the relationship between the speed and the general  
305 health of the subjects<sup>51</sup>.

306 The positive correlation between TDI and Swing Time CV suggested that the trunk displacement  
307 changes are directly proportional to the variability of gait. We cannot prove the directionality of the  
308 implication, but one can speculate according to the following line of reasoning. Since the balance  
309 control employed by the cerebellum acts on the COM, gathering vestibule-spinal information of  
310 trunk verticality<sup>32,35</sup>, it could be hypothesized that an impaired balance control of the upper body,  
311 as captured by increased TDI, which contains information of both trunk and COM, could determine  
312 instability during both the stance and swing phases of gait. Again, Osoba et al., reported increased  
313 gait variability associated with ageing and risk of fall<sup>50</sup>.

314

## 315 **5. CONCLUSIONS**

316 Our study shows that different RAS can influence the gait parameters in a frequency-specific  
317 manner, which means that a frequency equal to the individual natural frequency improve the  
318 stability. While low frequencies shows a general slowing down of gait, which do not provide any  
319 clear beneficial effect in terms of stability, an excessive low stimulation (80 bpm) seems detrimental  
320 to stability, being too slow compared to the individual natural frequency. In accordance with our  
321 expectations, using a frequency that exceeds a threshold value causes a worsening of the gait  
322 parameters and stability.

323 The high frequencies of RAS however provokes a slight speeding up of gait, which is  
324 accompanied by improved stability. Importantly, our study shows that a moderate increase of Speed  
325 is important to positively influence stability. In this case, fixed and variable RAS shared similar  
326 frequencies, hence no difference was found in gait with 110% AC and 120 bpm. This result supports  
327 our hypothesis that the effectiveness of the stimulation on stability depends on how far the stimulus  
328 is from the individual average cadence.

329 Eventually, the increased stability offered by the RAS may be used both in rehabilitation  
330 protocols and prevention strategies. RAS could support classical rehabilitation, adding a sensory  
331 training to the purely motor one, tailoring the quality and quantity of the stimulation on each  
332 individual. Moreover, the prevention techniques could be aided by the use of devices based on the  
333 RAS in order to support the gait continuously, increasing the stability of the individual and  
334 preventing them from falls.

335 One of the limitations of this study is the small data sample, due to the difficulty in finding and  
336 recruiting cognitively and physically healthy elderly subjects. Another one, is that the two high  
337 frequencies were quite similar to each other's, hence we could not explore the effects of a  
338 stimulation with a frequency much higher than the average cadence of each subject, as for the low  
339 frequencies. Future researches on the effects of RAS on the gait could aim to identify the threshold  
340 values above which there is a worsening of the gait and a reduction in stability.

341

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345

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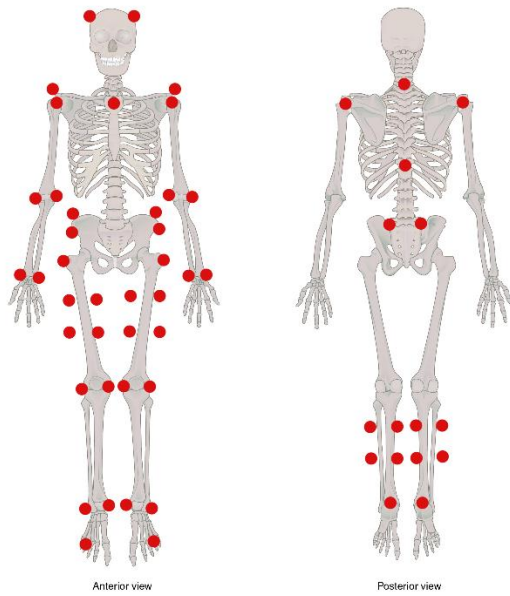
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<i>Participant Characteristics</i>	
<i>Demographic Data</i>	
<i>Ages (years)</i>	73.4 ( $\pm 5.7$ )
<i>Education (years)</i>	11.6 ( $\pm 4.8$ )
<i>Gender (m/f Ratio)</i>	6/7
<i>Anthropometric Parameters</i>	
<i>Weights</i>	65.27 ( $\pm 7.57$ )
<i>Heights</i>	161.78 ( $\pm 8.89$ )
<i>BMI</i>	25.0 ( $\pm 2.8$ )
<i>Neuropsychological Evaluation</i>	
<i>MMSE (adjusted)</i>	27.65 ( $\pm 1.85$ )
<i>FAB</i>	16.41 ( $\pm 1.66$ )
<i>BDI</i>	6.85 ( $\pm 3.98$ )

**Table 1.** Demographic, anthropometric and neuropsychological participant' characteristics (mean  $\pm$  standard deviation). Weights in kilograms, heights in centimetres, body mass index (BMI), mini mental state examination (MMSE), frontal assessment battery (FAB), Beck's depression inventory (BDI).

<i>Participants</i>	<i>100% AC frequency (bpm)</i>	<i>90% AC Frequency (bpm)</i>	<i>110% AC Frequency (bpm)</i>
1	115	103	126
2	117	104	128
3	106	95	117
4	113	102	124
5	105	95	115
6	90	81	99
7	109	98	120
8	129	116	142
9	110	99	121
10	100	90	110
11	108	97	119
12	119	107	131
13	112	100	123
	110.23 ( $\pm 9.46$ )	99 ( $\pm 8.40$ )	121.15 ( $\pm 10.37$ )

**Table 2.** Frequencies based on average cadence of each participant measured in beats per minute (mean  $\pm$  standard deviation). The 100%AC is the frequency equal to the average cadence of each participant. The 90% AC is the frequency equal to 90% of the participants' cadence. The 110% AC is the frequency equal to 110% of the participants' cadence.



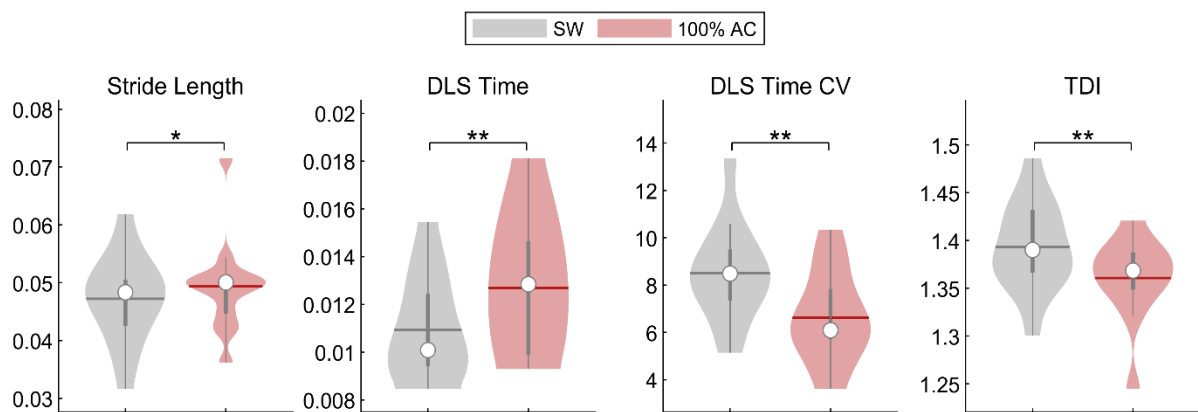
**Fig. 1** - Marker set of the fifty-five markers positioned on the anatomical landmarks of the feet, lower limb joints, pelvis, trunk, upper limb joints and on the head.

Adapted from "Axial and Appendicular Skeleton" (<http://cnx.org/content/col11496/1.6/>) by OpenStax College, used under CC by 4.0 (<https://creativecommons.org/licenses/by/4.0/>)

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**Fig. 2** - Spatio-temporal analysis of gait and Trunk Displacement Index (TDI). SW vs. 100% AC

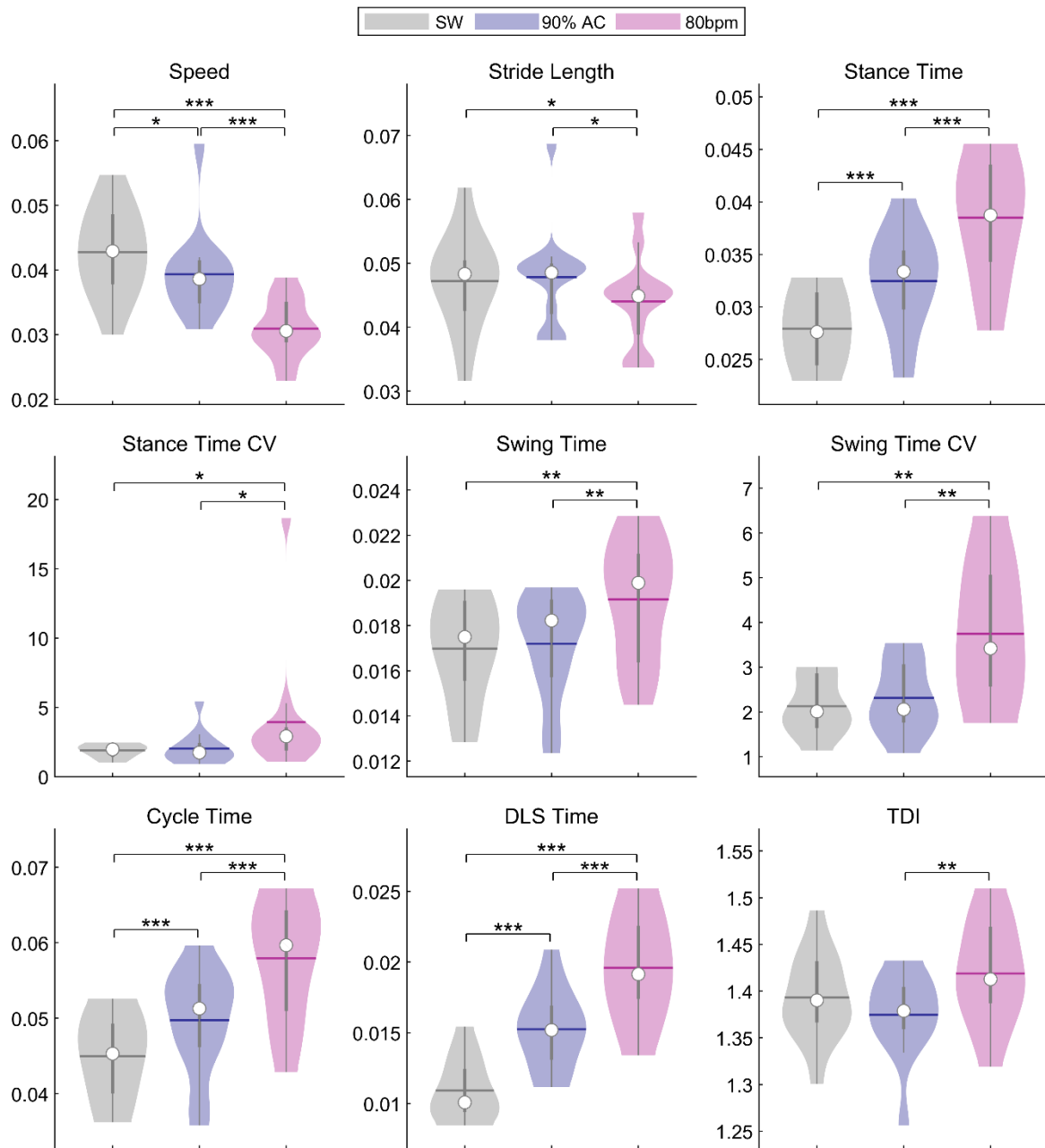
478 Violin plots of spatio-temporal parameters and TDI value. Comparison between simple walking (SW) and  
479 walking with RAS at frequency equal to the subject-specific average cadence (100% AC). Stride Length;  
480 Double Limb Support Time (DLS Time); Trunk Displacement Index (TDI); Coefficient of Variability of Double  
481 Limb Support Time (DLS Time CV); Significance p value: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

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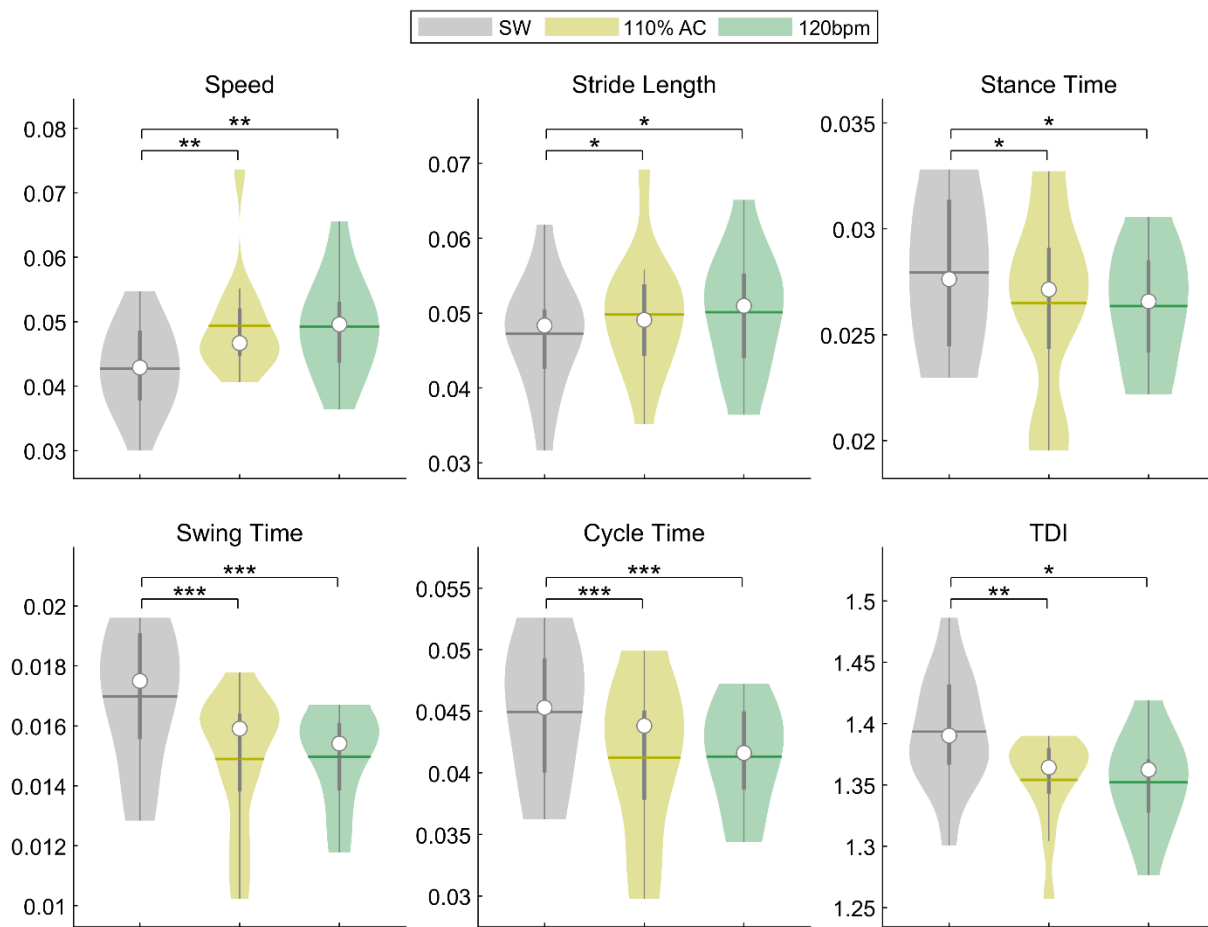
487 **Fig. 3 - Spatio-temporal analysis of gait and Trunk Displacement Index (TDI). SW vs. Low Frequencies RAS**

488 Violin plots of spatio-temporal parameters and TDI value. Comparison between simple walking (SW) and  
489 walking with RAS at low frequencies (SW - 90% AC - 80 bpm). Speed; Stride Length; Stance Time; Coefficient  
490 of Variability of Stance time (Stance Time CV); Swing time; Coefficient of Variability of Swing time (Swing Time  
491 CV); Cycle Time; Double Limb Support Time (DLS Time); Trunk Displacement Index (TDI); Coefficient of  
492 Variability of Double Limb Support Time (DLS Time CV); Significance p value: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p <$   
493  $0.001$ .

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498 **Fig. 4 - Spatio-temporal analysis of gait and Trunk Displacement Index (TDI). SW vs. High Frequencies RAS**

499 Violin plots of spatio-temporal parameters and TDI value. Comparison between simple walking (SW) and  
500 walking with RAS at high frequencies (SW - 110% AC – 120bpm). Speed; Stride Length; Stance Time; Swing  
501 time; Cycle Time; Trunk Displacement Index (TDI); Coefficient of Variability of Double Limb Support Time (DLS  
502 Time CV); Significance p value: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

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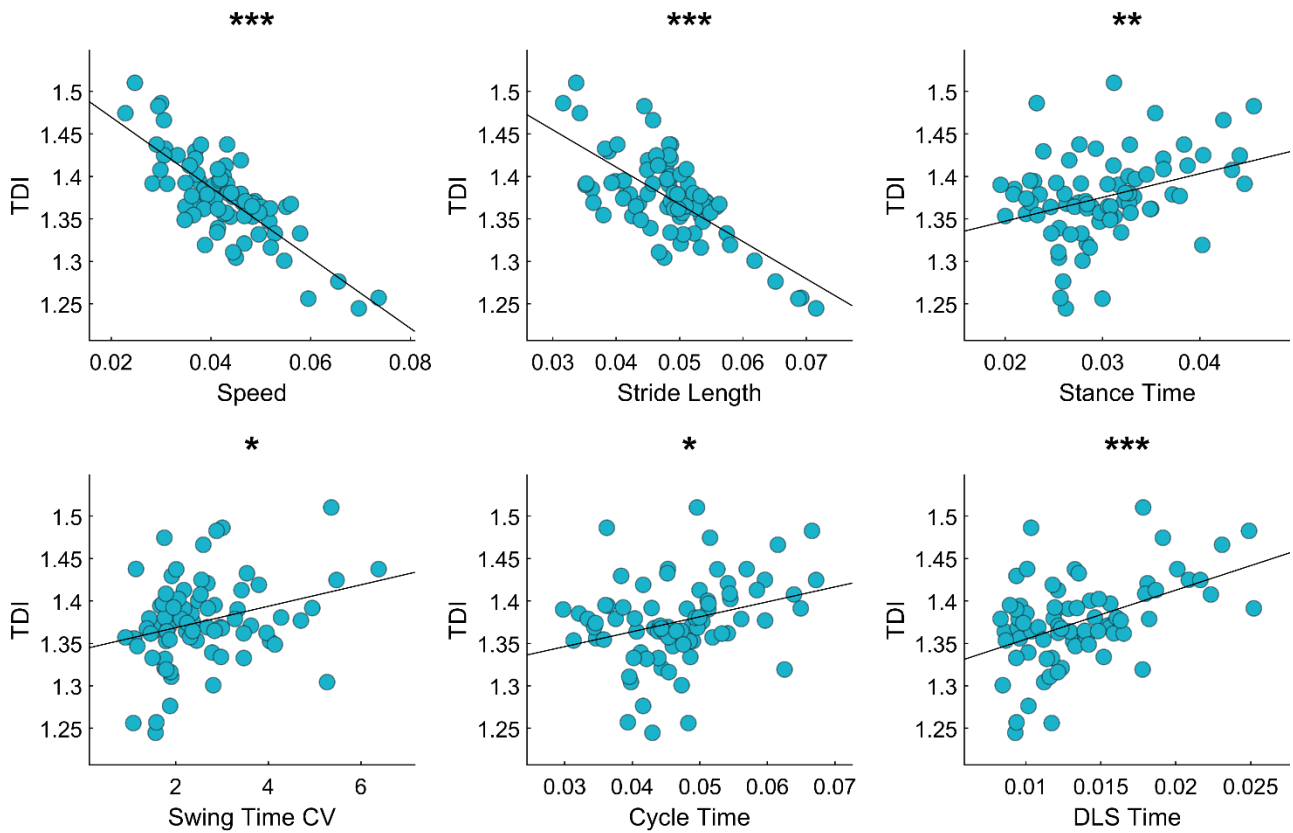
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512 **Fig 5. TDI and spatio-temporal gait parameters.**

513 Pearson coefficient correlation between trunk displacement index (TDI) and spatio-temporal gait parameters  
514 in all conditions. Speed, Stride length, Stance time, Coefficient of variability of Swing Time (Swing Time CV),  
515 Cycle Time. Significance p value: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

516