ANCESTRAL PERSISTENCE OF VESTIBULO-SPINAL REFLEXES IN AXIAL MUSCLES IN HUMANS

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ABSTRACT

Accurate control of the trunk is essential for maintaining balance in upright subjects. Most studies addressing vestibulo-spinal reflexes have investigated the role of the lower limbs, while limited attention has been paid to the back muscles. To address this issue, we challenged the persistence of vestibular evoked myogenic potentials (VEMPs) in back muscles in situations in which the leg muscle responses were modulated. Nineteen subjects were submitted to galvanic vestibular stimulations (GVS). Body sway and VEMPs were recorded in the paraspinal and limb muscles. During treadmill locomotion, the VEMPS in the lower limbs were observed only during the stance phase, whereas the axial VEMPs were observed during all phases. In upright standing subjects, slight head contact was sufficient to abolish the VEMPs in the lower limbs, while the VEMPs remained present in the paraspinal muscles. Similarly, during parabolic flight-induced microgravity, the VEMPs in the lower limb muscles were suppressed, while those in the axial muscles persisted despite the absence of gravitational information from the otolithic system. Our results depict a differentiated control mechanism of axial and appendicular muscles when a perturbation is detected by vestibular inputs. The persistent feature of axial myogenic adjustments suggests that a hard-wired reflex is functionally efficient to maintain posture. By contrast, the ankle responses to perturbations occur only when the accompanying sensory feedback is congruent, challenging the balance task and gravity. Overall, this study using GVS in microgravity is the first to present an approach delineating feed-forward vestibular control in the absence of all feedback.

39 **NEW AND NOTEWORTHY**

40 We addressed the degree to which the underlying neural circuitry and mechanisms
41 involved in trunk muscle control have been preserved in human. We highlighted the
42 persistence of vestibular-evoked responses in trunk muscles using galvanic vestibular
43 stimulation, in conditions where leg muscle responses were modulated (walking, standing,
44 microgravity). This suggest a phylogenetically conserved blueprint of sensorimotor
45 organization, with strongly hard-wired vestibulo-spinal inputs to axial motoneurons and a
46 higher degree of flexibility for the late emerging limb.

INTRODUCTION

During evolution, locomotion has adapted from propulsion by undulation to quadrupedal and finally bipedal propulsion. Despite its decisive advantages, an erect posture strengthens the difficulty of maintaining balance. This ability to adjust the body orientation, particularly during dynamic tasks, highly relies on the vestibular system. This sensorial input provides adequate information related to head movement and orientation and may evoke responses in limb muscles and postural adjustments (Britton et al., 1993; Fitzpatrick et al., 1994). Despite their high degree of automaticity, vestibulomotor responses are extremely flexible and may vary in a task-dependent manner. Various studies have demonstrated that motor responses in the upper and lower limbs following the vestibular detection of motion directly depend on limb engagement in the current task. For instance, using galvanic vestibular stimulation (GVS, Goldberg et al., 1984; Fitzpatrick and Day 2004), arm motor responses were observed during reaching (Bresciani et al., 2002; Mars et al., 2003; Smith and Reynolds, 2017) or body stabilization when the hand is used to help maintain balance (Britton et al., 1993). This response was absent in the contralateral arm, which was not engaged in the task (Britton et al. 1993). Comparable results have been observed in the lower limb muscles since in seated subjects or subjects standing upright while bearing a support, vestibular evoked responses were no longer present in the leg muscles (Fitzpatrick et al., 1994). A recent study (Forbes et al., 2016) showed that a reverse relationship exists between balancing motor command and associated vestibular sensory feedback in a task conducted in the reverse direction of the vestibular evoked compensatory response. The flexibility of vestibulo-spinal processes has also been documented during walking (Bent et al., 2004; Iles et al., 2007; Forbes et al., 2017; Blouin et al. 2011). Altogether, these findings suggest that new relationships between vestibular signals and consequent motor commands can be established according to the context, even in similar tasks.

However, most studies addressing the control of balance in upright subjects have mainly investigated the role of the lower limbs, while limited attention has been paid to the vestibulo-spinal reflexes that occur in the back muscles. This is surprising because the trunk, which represents more than 60% of the total body mass and is located above the center of gravity, is a potential source of major instability. Because this body part is highly articulated and actuated by many muscles, it should be accurately controlled during displacement. Ali and

colleagues (2003) recorded functional vestibular evoked responses in the erector spinae muscles in both sitting and upright postures, and any response was measured in the limb muscles in the sitting posture. Interestingly, the subjects' responses in the erector spinae recorded at L3/L4 displayed latencies of 59 ms, while the latencies in the lower limb muscles were much longer (≈85 ms). These results suggest that muscles of the axial trunk and leg may be controlled by different vestibulo-spinal mechanisms.

Therefore, in this paper, our objective was to challenge the persistence of vestibular evoked responses in the back muscles under conditions in which the lower-body muscle responses are modulated by a task and/or context using GVS. In the first experiment, we compared the GVS phase effect dependency on back muscles and lower limb muscles during walking. In the second experiment, we tested two conditions in which vestibular evoked myogenic potentials (VEMPs) were suppressed in the lower limbs, i.e., in upright subjects bearing a support and upright subjects sustained in a harness. In the third experiment, which represented our "most challenging" condition, we tested the persistence of vestibulo-spinal reflexes in floating subjects in the absence of gravity (during parabolic flight) as no postural adjustments were required. To the best of our knowledge, this study represents the first time GVS responses were studied in microgravity. Altogether, we demonstrate that under all these contextual and/or gravitational conditions, balance and posture were persistently controlled using back muscles whereas the lower limb responses could be flexibly modulated.

MATERIAL AND METHODS

Nineteen adults participated in this study. The subjects provided written informed consent prior to participating in each experiment. The procedures conformed to the Declaration of Helsinki and were approved by the French National Research Ethics Committee (Comité de Protection des Personnes Sud-Ouest et Outre Mer III) under agreement number 2011-A00424-37. In all experiments, we used similar tools and methods, including pulse GVS stimulation, electromyography and 3D kinematics.

Vestibular stimulations

106 Bipolar binaural galvanic electrical stimulations were applied to the mastoid processes.
107 The electrodes were circular with a contact surface of 9 cm^2 (Axelgaard Pals Platinium, Ø32
108 mm). Square pulse stimulations (3.5 mA; 175 ms) were delivered using an isolated constant
109 current stimulator (Digitimer DS-5, CE mark certification for medical devices) with randomized
110 and unpredictable delays (5 s minimum between two stimulations).

111 **Electromyographic recordings and analysis**

112 EMGs were recorded bilaterally on the *medial gastrocnemius*, *anterior tibialis*, and
113 *erector spinae* at the C7, T7 and L4 levels (De Sèze et al., 2008; Cecatto et al., 2009). During
114 walking on a treadmill, we used a wireless EMG system (Kine ehf, Iceland; 1.562 KHz, x10,000
115 amplifier), while all other recordings were performed using an analogical amplifier (TeleEMG,
116 BTS, Milano, Italy; x1000 amplifier) linked to an ITC-18 A/D interface (Heka, Lambrecht,
117 Germany; 2KHz). The same A/D card was used to start the GVS and simultaneously record the
118 EMG signals (except for during the walking experiment).

119 From the EMG signals from the erector spinae, the GVS artifacts (shifts due to voltage)
120 were removed *a posteriori* by digitized data shift compensation. During the walking
121 experiment, the presence of GVS artifacts on the EMG traces (voltage shift) was automatically
122 detected (and then controlled for by subsequent visual inspection) on the left C7 erector
123 spinae signal (the recorded muscle nearest the GVS electrode). During the other experiments,
124 we simultaneously recorded EMG and a copy of the GVS stimulator output to precisely and
125 unambiguously identify the GVS artifacts. For each EMG channel, the drift observed in the 40
126 ms following the GVS onset was modeled by a third order polynomial and removed from the
127 signal. The same method was simultaneously applied to remove the artifact at the end of GVS.
128 Then, the EMGs were numerically high-pass filtered (zero-phase Butterworth filter at 30 Hz),
129 rectified, and smoothed with a 20 samples moving average.

130 For each stimulation, a 4 s time window was defined on both sides of the GVS onset (2
131 s before and 2 s after). For each subject, the EMGs were subsequently synchronized and
132 averaged for each GVS polarity and condition and normalized by the activity measured before
133 the occurrence of GVS (tonic activity during normal standing = 100%). The detection threshold
134 to identifying VEMPs during the 300 ms following the GVS start was two standard deviations
135 from the baseline. The baseline was defined as the median EMG during the 500 ms that

136 preceded the GVS start. The medium latency of the VEMPs (ML; Fig. 4; Britton et al., 1993)
137 was visually identified by comparing the EMGs following GVS to those at baseline or
138 comparing the EMGs following GVS to those from opposed polarities if any.

139 The area of the VEMPs was computed by trapezoidal numerical integration of the
140 rectified EMGs. The portions of EMGs used to compute the areas for each muscle in each
141 subject in each experiment were the portions between the starting and ending time of the
142 identified VEMPs averaged across all subjects. Then, we could compute the EMG areas in
143 portions where VEMPs were expected, even if VEMPs were absent, allowing a between
144 conditions comparison.

145 **Center of pressure**

146 Static balance was assessed in a laboratory using a force plate (AMTI BP600900 with 6
147 degrees of freedom), and the position of the center of pressure was recorded at a 50 Hz
148 sampling frequency. The data from the force plate were synchronized to the GVS start and
149 averaged by subject, condition and GVS polarity.

150 **Kinematic recordings**

151 We used a 3D-motion analysis system for the body segment analysis (Natural Point,
152 Corvallis, USA). Twelve Optitrack Flex3 (640 × 480 pixels, 100 Hz) cameras were used in the
153 gait experiment, and five Optitrack S250e (832 × 832 pixels, 250 Hz) cameras were used in the
154 parabolic flight experiment. Prior to the data collection, calibration was performed to
155 determine the camera orientation in relation to the working volume and the relative position
156 of each camera to the other camera. Spherical reflecting passive markers (15 mm in diameter)
157 were positioned on the head, trunk and body segments (see below). The data acquisition was
158 performed using custom built software developed in MATLAB R2013a (MathWorks, Natick,
159 MA, USA), enabling the simultaneous collection of synchronized kinematic and EMG data.

160

161 **Experiment 1: GVS during walking**

162 Eleven subjects participated in the experiment (5 females and 6 males, 29.1 [±7] years
163 old). The subjects walked on a treadmill (Pulsar 4.0, HP-Cosmos, Nußdorf Germany) at 0.75

164 m.s^{-1} with their eyes closed and gaze straight ahead. The subjects performed a 10-minute
165 training before the first GVS stimulation. The subjects were secured by a harness, and the
166 treadmill automatically stopped in case of falls in the harness (which never occurred). Each
167 subject performed 12 trials, and each trial lasted 90 s with 5-minute pauses between each
168 trial. During the experiment, each subject received more than 160 GVS (inter-subject
169 mean=172) with unpredictable delay (>6s) and pseudo-randomized laterality (anode right or
170 left).

171 We placed three reflective markers on each of the following segments: head, scapular
172 girdle, pelvic girdle, hands, and feet. A real-time kinematic measurement of foot
173 displacements was performed using a custom software developed with MATLAB to determine
174 the phase of the gait cycle during which GVS occurred. Using this setup, we detected heel
175 strikes based on the antero-posterior velocity of each foot. On the treadmill, the foot velocity
176 shifted from positive to negative at the heel strike. GVS could be delivered at this time event
177 with a latency as short as 40 ms (approximately 3% of the gait cycle). For each subject, the
178 average gait cycle duration was determined during a ten-minute training, allowing GVS to be
179 applied during the following four different phases of the cycle: (1) the first double support
180 phase (immediately after the right heel strike); (2) the left swing (15% after the right heel
181 strike); (3) the second double support phase (immediately after the left heel strike); and (4)
182 the right swing (15% after the left heel strike). During the data analysis, each locomotor cycle
183 was identified based on the right heel strike. During the post hoc analysis, we checked that
184 GVS occurred at the proper phase.

185 To compare the effects of GVS during different phases of the gait cycle, we
186 synchronized all kinematic signals to the GVS start, and the data were averaged to obtain one
187 measure per subject, segment, cycle phase and GVS polarity. The anode right vs. left GVS
188 induced medio-lateral deviations in opposite directions with the same delay. We compared
189 the averaged medio-lateral velocities at both polarities to visually identify the latencies of the
190 kinematic deviation. The maximum magnitude of the medio-lateral deviation was measured
191 during the 500 ms following the GVS start.

192 The EMG signals were analyzed by performing a cycle categorization according to the
193 phase of GVS occurrence (right heel strike, left swing, left heel strike, right swing, or no GVS).

194 To analyze the phase effect on the VEMPs, the data were synchronized and averaged to obtain
195 one sample synchronized to the GVS start per subject, muscle, and phase of the cycle. Finally,
196 the EMGs of each muscle from each subject were normalized and expressed as a percent of
197 the median activity during the No-GVS gait cycles. The occurrence and latencies of the VEMPs
198 were visually identified by comparing the EMGs following GVS at opposite polarities.

199 **Experiment 2. GVS during upright standing with or without upper body contact**

200 Nine subjects participated in this experiment (5 females and 4 males, 25.3 [±4] years
201 old). We measured the effects of GVS on upright standing subjects under the following two
202 conditions: (1) "freestanding" in which the subjects had to stay upright with their head turned
203 to the right and their eyes closed and (2) "head contact" in which the subjects remained in the
204 same position but with their head slightly in contact with a wall mounted on wheels allowing
205 for precise proprioceptive feedback regarding body sway but could not be used as a firm
206 support. Each subject was submitted to bipolar GVS with the anodal stimulation randomly set
207 on the left or right mastoid process. Each condition included 20 trials, and each trial included
208 4 GVS with randomized polarity and an unpredictable delay (>5 s). The condition occurrence
209 was randomized throughout the test session. Each subject experienced 160 GVS (2 conditions
210 x 20 trials x 4 stimulations).

211 **Experiment 3. GVS in the postural muscle of free-floating subjects**

212 This experiment occurred in the Zero-G airplane (A300, Novespace, Mérignac, France)
213 chartered by the Centre National d'Etudes Spatiales (CNES, France) during two parabolic flight
214 campaigns (VP-92 and VP-95, 2011-2012; 6 flights in total). During each flight, 30 parabolas
215 were performed, and two subjects were tested (15 parabolas per subject). Each parabola
216 provided a time lapse of 22 s of zero gravity (Fig. 5A), which was preceded and followed by 20
217 s time lapse of increased gravity (1.8 g; Fisk et al. 1993). Due to the influence of scopolamine
218 on motor control (Bestaven et al., 2016), the subjects were not medicated.

219 Twelve subjects were tested in the upright standing position with their heads turned
220 to the right, anode on the posterior mastoid process, and vision occluded by a mask. GVS
221 occurred during normogravity (4 stimulations during each steady flight period between the
222 parabolas), the first hyper-gravity period (n=4), and micro-gravity (n=4), resulting in a total of

180 GVS. All GVS were delivered at the same polarity to trigger VEMPs in the gastrocnemius and back muscles (anode posterior). Because the feet usually leave the floor in micro-gravity, the subjects did not have any contact with their environment and were secured. Three conditions were tested. Under the first condition, designated "Free-floating", a scapular harness on the upper body secured the subjects (n=6) with distended tether (Fig. 5A). This harness did not apply any force on the subject, except for in the case of exceptional excursion (usually prevented by an operator situated in front of the subject during the experiment). The results of the first campaign (i.e., microgravity VEMPs remained present in the trunk only) prompted us to add other conditions to confirm that the observed effects were not due to (1) the presence of the harness on the upper body and/or (2) the lack of feet contact and external support. Thus, during the second campaign (VP95), the subjects (n=6) were tested under a second condition designated "Foot-strapped". The subjects were submitted to different gravity conditions without a harness while being efficiently linked to the floor with their feet strapped to soft pads. The straps and pads we used (Concept X Kitesurf) allowed the ankle to be actuated in all directions with very good proprioceptive feedback. The same stimulation and recording protocols were performed during the first and the second campaign.

The third condition, i.e., “Harness”, was a control condition that was performed at our laboratory to consider the influence of gravitational detection and/or spine loading on the VEMPs of the lower limbs. In this experiment, the subjects were sustained in an upright position in a pelvian harness with their feet 10 cm above the floor. As in microgravity, the subjects stayed upright with an unloaded lower limb, but proprioceptive, visceral and otolithic perception of the gravitational field was present. The subjects also had their heads turned to the right, their vision was occluded, and they received 40 GVS with the anode on the posterior mastoid process and unpredictable delays. The same subjects (n=9) were tested under the standing upright (experiment 2) and Harness conditions.

Kinematics

We placed three reflective markers on each of the following segments: head, scapular girdle, and pelvic girdle. The 3D data were captured by 5 cameras (Optitrack s250e) at 250 Hz. The EMG data were synchronized and averaged to obtain one sample synchronized to the GVS start per subject, segment, Gravity and condition. Based on the averaged data, we manually

identified the latencies of the GVS induced deviation based on the antero-posterior displacement velocity (sagittal plane). We computed the maximal posterior sway during the 1500 ms following the GVS start.

Statistical analysis

For each experiment, a repeated-measures analysis of variance was performed using the MATLAB R2017a "ranova" function, and a post hoc analysis was performed using the comparison test of Scheffé. ANOVAs were computed to analyze the EMG area and latencies and kinematic magnitude and latencies. For each experiment, all conditions were considered within factor with repeated measures, except for the Free-floating vs. Foot-strapped conditions, which were considered between-factor because not all subjects participated in both campaigns (one condition per campaign). To statistically compare the proportion of VEMP occurrence, a Z-test was computed.

RESULTS

Experiment 1: Effects of bilateral GVS pulse during gait

This experiment was designed to investigate whether there was a phase dependency on vestibulo-spinal inputs during gait cycles and whether vestibulo-spinal inputs to axial or lower limb muscles were differentially controlled during walking. The subjects maintained their gaze straight ahead with their eyes closed, and GVS created a roll illusion contralateral to the anode, which elicited head displacement ipsilateral to the anode (Fig. 2A). Such lateral displacements were observed in the upper body segments (head, scapula and pelvis) and the stance foot (grounded during GVS but deviated at the following step). By contrast, the swing foot was deviated in the direction opposite to the upper body parts contralateral to the anode. The displacement magnitude in the frontal plane in the more rostral parts was higher (Fig. 2B; head: 6.9 cm (SD .9); scapular girdle: 6.1 cm (SD .8); pelvic girdle: 3.9 cm (SD .6)). The feet were deviated with a magnitude of 2.6 cm (SD .8) in the swing foot and 6.1 cm (SD 1.2) in the stance foot. The opposite directions of deviation between the upper parts of the body and swing foot along with the increased deviation observed in the more cranial parts elicits a body rotation in the frontal plane with an axis located at the hip level.

As expected, we found an effect of the anode side (i.e., polarity) and cycle phase, resulting in a significant interaction among Segment x Polarity x Phase ($F(12,48)=23.6$, $p<0.001$). The post hoc analysis revealed that the magnitude of the deviation of the head, scapular and pelvic girdles did not depend on the cycle phase at which GVS was delivered. By contrast, we found significant differences (Scheffe with $p<0.05$) in the feet displacements according to the cycle phase at which GVS was delivered. More specifically, in the right foot, the GVS effect during the first double stance phase did not differ from the left swing (both loaded phases), and the GVS effect during the second double stance phase did not differ from the right swing (both unloaded phases). However, each loaded phase (first double stance and left swing) significantly differed from each of the unloaded phases (second double stance and right swing). The same findings were observed in the left foot with no difference between the first double stance and left swing (loaded phases), no difference between the second double stance and right swing (unloaded phases), and significant differences between phases originating from each of these two groups (loaded vs. unloaded). This effect was observed in both GVS polarities (anode on the right or left side) but with opposite deviations.

The latency values of the kinematic deviations differed in specific body parts according to the phase (significant Segment x Phase interaction, $F(12,48)=5.3$, $p<0.001$), but the post hoc analysis revealed significant differences in the feet only. When GVS was delivered, a deviation occurred in the grounded foot during the following toe off. Consequently, in both feet, the deviation latencies during the loaded phases (swing of the contralateral foot, 533 ms (SD 127)) were significantly higher than those during the swing phases (214 ms (SD 47)). Similarly, the deviation latencies of the grounded foot were significantly higher than those of the head (203 ms (SD 135)) in the same phase but did not significantly differ from the latencies of the pelvic girdle (343 ms (SD 113)) and scapular girdle (333 ms (SD 105)). Significant differences were not observed among the head, scapular girdle, pelvic girdle and swinging foot deviation latencies.

The muscle activities observed during walking without GVS are consistent with previously observed activities on a treadmill (DeSeze et al., 2008; see Fig. 3 A). The erector spinae at T7 and L4 present a double burst rhythmic activity recorded in the right and left side with one burst after each heel strike. The gastrocnemius only presented one burst per cycle at the end of the stance phase (when the triceps surae is propulsive). To determine the GVS

313 effects, we measured the EMG area and the latency value of the response immediately
314 following GVS. As the full statistical plan was 7 muscle levels x 2 sides x 4 gait phases x 2 GVS
315 polarities, we simplified the model by averaging the right and left muscles at each level with
316 respect to the GVS polarity and loaded foot laterality. For instance, right ES L4 with right anode
317 GVS and left foot loaded was averaged with left ES L4 with left anode GVS and right foot loaded
318 to obtain ES L4 activity with "GVS ipsilateral" and "stance contralateral". This resulted in the
319 statistical plan 7 muscles x 2 GVS polarities (Ipsi/Contra) x 2 phases (Loaded/Unloaded side).

320 Interestingly, we did not observe significant effects of GVS polarity on the VEMP areas
321 of *ES* at the C7 and T7 levels in the *rectus abdominis*, *pectoralis major* and *tibialis anterior*. An
322 effect of the side of the loaded foot was observed in *rectus abdominis* ($F(1,10)=5$, $p<0.05$,
323 Ipsi>Contra) and was near significant at ES C7 ($F(1,10)=4.1$, $p=0.07$, Contra>Ipsi). A significant
324 effect of the GVS polarity was observed in EMG area ES L4 (Fig. 3B upper panel) with higher
325 muscle activities following ipsilateral GVS than contralateral GVS ($F(1,10)=15.1$, $p=0.003$) and
326 no loading phase effect. In *gastrocnemius*, a significant interaction between GVS polarity and
327 the loading phase was observed ($F(1,10)=34.5$, $p<0.001$, Fig. 3B lower panel). The post hoc
328 analysis revealed an effect of GVS polarity (Contra>Ipsi) in the gastrocnemius area only during
329 ipsilateral foot loading.

330 Regarding the VEMP latencies, we compared the onset of the VEMPs elicited by
331 contralateral GVS to ipsilateral GVS (VEMPs with the same latencies but opposite effects on
332 the EMG magnitude). This analysis was performed at the ES T7, L4, Gastrocnemius and TA
333 muscles, where the effects of the opposed GVS were easily identifiable. The two-way ANOVA
334 (4 muscles x 2 phases) showed a main effect of muscle only ($F(3,9)=67$, $p<0.001$) on the VEMP
335 latencies. The post hoc analysis revealed that the erector spinae at T7 and L4 did not have
336 significantly different latencies (34 ms (SD 7) and 41 ms (SD 10), respectively), while these
337 muscle latencies were significantly shorter than the lower muscles latencies at gastrocnemius
338 and TA (140 ms (SD 18) and 173 ms (SD 30), respectively, not different). Altogether, these
339 results indicate that the GVS effect is strongly phase dependent in gastrocnemius but not
340 modulated by the gait cycle in the upper part of the body.

341

342 **Effects of GVS on upright standing subjects**

343 It has been shown that in seated subjects or subjects standing upright while bearing a
344 support, vestibular evoked responses were no longer elicited in the leg muscles (Fitzpatrick et
345 al., 1994). In the present experiment, we investigated whether the same sensory gating of
346 vestibulo-spinal inputs occurred in a similar way in the axial and leg muscles. Therefore, we
347 compared the GVS-induced responses in the absence or presence of a contact with support.
348 A bilateral GVS with the anode on the right (anode back) or left (anode front) mastoid process
349 was delivered with the head turned to the right and occluded vision. The GVS with the anode
350 back induced a backward sway (Fig. 4A) with an activation of the erector spinae and
351 gastrocnemius muscles (Fig. 4BC). The maximum amplitude of the center of pressure during
352 this antero-posterior sway was reduced by head contact (-2.9 mm (SD .9) in freestanding and
353 -.2 mm (SD 1.48) with head contact; N= 9 subjects; Fig. 4A left vs. right panel). The two-way
354 ANOVA revealed a significant interaction between the effects of polarity and condition on the
355 antero-posterior magnitudes of the COP excursions ($F(1,6)=18.3$, $p=0.005$). The forward sway
356 induced by GVS with the anode-front was also reduced (1.4 mm (SD 1.2) in freestanding and
357 1.2 mm (SD 1.41) with head contact), but this effect was not significant. The delay of the
358 occurrence of the maximum sway was not significantly affected by either the polarity or head
359 contact with a mean value of 379 ms (SD 60).

360 Several responses were elicited by GVS in the recorded muscles. As previously reported
361 (Nashner et al. 1974; Britton et al. 1993; Fitzpatrick et al. 1994), GVS with the anode back first
362 elicited a response with a short latency (single arrows, Fig. 4BC left panel) in the erector spinae
363 and gastrocnemius muscles and a medium latency response (double arrow, Fig. 4BC). The
364 short- and medium-latency EMG responses were in opposite directions at the lower limb level
365 only but not in ES. We focused on the medium latency response, which was larger in
366 amplitude, and its direction was correlated with the observed pattern of whole body sway.
367 The comparisons of the VEMP occurrences indicates that the contact condition only
368 influenced the gastrocnemius response. The GVS-elicited VEMPs from the erector spinae (L4
369 level; Fig. 4B) were detected in 78% of the subjects under the freestanding-condition, and this
370 proportion was not significantly reduced to 56% under the head-contact condition ($Z=1$,
371 $p=0.16$). The GVS-elicited VEMPs from the gastrocnemius were detected in 100% of the
372 subjects under the freestanding-condition (Fig. 4B), but this proportion was significantly
373 reduced to 22% of the subjects under the head-contact condition ($Z=3.38$, $p<0.001$).

374 In the erector spinae (L4), the VEMP area was affected by the GVS polarity. As
375 previously described, GVS with the anode back (Fig. 4B) elicited a short latency inhibitory
376 response and a medium latency response of a large amplitude. By contrast, when GVS was
377 delivered with the anode front, the medium latency response was dramatically decreased.
378 The two-way ANOVA revealed a significant main effect of polarity on the erector spinae
379 elicited VEMP area ($F(1,8)=8.5$, $p<0.05$) and no effect of condition or interaction. Interestingly,
380 the same analysis of the VEMPs elicited in the gastrocnemius revealed an interaction between
381 the GVS polarity and contact condition ($F(1,8)=7$, $p<0.05$). The post hoc analysis showed a
382 strong effect of the GVS polarity under the freestanding condition with a large response in the
383 gastrocnemius to the anode back GVS and a marked inhibition with the anode-front GVS (Fig.
384 4C). This effect was drastically and significantly reduced under the head-contact condition
385 with responses representing only 28% of the VEMP areas recorded while freestanding.

386 The VEMP latencies were measured at ES-L4 and the gastrocnemius under the
387 Freestanding condition. The repeated-measures ANOVA revealed significantly shorter
388 latencies in the erector spinae (49 ms (SD 26)) compared with those in the gastrocnemius (89
389 ms (SD 16); $F(1,6)=11.7$, $p<0.05$). The latencies at ES were not affected by the head contact
390 ($F(1,5)=0.26$, $p=0.63$; 51 ms (SD 22) in free standing and 47 ms (SD 35) with head contact).

391 Altogether, these results revealed that incongruent proprioceptive cues had a major
392 effect on the VEMPs in the gastrocnemius but not ES back muscles.

393

394 **Effects of GVS in modified gravity**

395 To challenge the functioning of the vestibulo-spinal system, we performed
396 experiments in which gravity was modified. Under microgravity, otolithic afferents are lacking
397 because their activation depends on the effects of gravity; hence, the postural adjustments
398 maintaining equilibrium become functionally useless.

399 Figure 5B present the experimental design of the free floating conditions (see
400 Materials and Methods section). The subjects were submitted to GVS during parabolic flights
401 (Fig. 5A) with periods of normal gravity, hyper-gravity (1.8 g) and micro-gravity (0 g). Due to
402 the limited number of available trials under these conditions, GVS was delivered with the

403 anode back only. GVS induced a backward displacement of the upper body in the antero-
404 posterior direction under the normal gravity conditions (Fig. 5C, solid line, sagittal plane),
405 which was increased under the hypergravity condition (dash-dotted line; $F(2,8)=2$, $p=0.19$ with
406 $n=4$ subjects) and disappeared under the microgravity condition (dotted line). Under the
407 normogravity and hypergravity conditions, all subjects expressed VEMPs in ES-L4 and
408 gastrocnemius, whereas under the microgravity condition, 100% of the subjects expressed
409 VEMPs in the left ES-L4, 60% of the subjects expressed VEMPs in the right ES-L4, and no
410 subjects expressed VEMPs in the gastrocnemius (significant decrease, $Z=5.2$, $p<0.001$). We
411 found a significant main effect of gravity on the ES-L4 muscle area (Fig. 6A1; $F(2,8)=6$, $p<0.05$
412 for left ES-L4 and $F(2,8)=5.8$, $p<0.05$ for right ES-L4). The post hoc analysis only revealed a
413 significant difference between the hypergravity and microgravity conditions in the left ES-L4
414 area. We also found a significant main effect of gravity on the gastrocnemius area in the free-
415 floating condition (Fig. 6A2; $F(2,8)=23.8$, $p<0.05$ for left gastrocnemius and $F(2,8)=25$, $p<0.05$
416 for right gastrocnemius). The post hoc analysis revealed that in both sides, the gastrocnemius
417 EMG area was drastically decreased under the microgravity condition (quite null) and
418 enhanced under the hypergravity condition (all p-values <0.05) compared with that under the
419 normogravity condition.

420 Finally, a one-way repeated-measures ANOVA of the latencies at ES-L4 under the free-
421 floating condition did not reveal any significant differences among the latencies at the three
422 levels of gravity (micro-, normo-, and hyper-gravity; $F(2,4)=3.1$, $p=0.15$; Table 2).

423 Under the foot-strapped condition, we tested GVS only in microgravity and
424 normogravity. Measuring the occurrence of VEMPs under the foot-strapped condition also
425 revealed that the VEMP occurrence remained stable at the ES-L4 level (from 86% in
426 normogravity to 75% in microgravity, $Z=0.54$, $p=0.29$ in ES-L4 right; from 100% to 86%, $Z=0.53$,
427 $p=0.29$ in ES-L4 left), while the VEMPs were totally abolished in the gastrocnemius in the
428 absence of gravity (from 100% to 0%, $Z=7.3$ and $p<0.001$ in both side). There was no significant
429 difference in the ES-L4 VEMP area between the normogravity and microgravity conditions
430 (Table 1; $F(1,6)=2.9$, $p=0.14$ in left ES-L4 and $F(1,6)=0.84$, $p=0.39$ in right ES-L4), whereas a
431 main effect of gravity on the gastrocnemius VEMP was observed (left gastrocnemius
432 $F(1,6)=10.3$, $p<0.05$; right gastrocnemius $F(1,6)=6.7$, $p<0.05$).

The repeated-measures ANOVA of the latencies with the condition (free-floating vs. foot-strapped) as a between subjects factor and muscle (ES-L4 vs. gastrocnemius) as the within subjects factor revealed a main effect of muscle ($F(1,8)=37$, $p<0.001$) with latencies at the ES-L4 (67 ms (SD 15)) that were much shorter than the latencies at the gastrocnemius (105 ms (SD 18)). Theses latencies were not significantly affected by the condition (see Table 2). Another two-way ANOVA was performed to analyze the latencies at ES-L4 only with the condition (free-floating vs. foot-strapped) as the between subjects factor and gravity (micro- vs. normo-) as the within subjects factor. Neither gravity nor the condition significantly influenced the latencies ($F(1,6)=5.4$, $p=0.06$).

Finally, we performed a laboratory experiment in which the subjects were suspended in a harness. The aim was to evaluate the role of sensory cues in the gating of gastrocnemius VEMPs with subjects in the upright position, which is comparable to free-floating but with gravitational otolithic activation and spine load. The proportion of subjects in which GVS elicited VEMPs remained the same in the left ES-L4 prior to and during suspension (83%; $Z=0$, $p=0.5$), while there was a non-significant decrease ($Z=0.67$, $p=0.25$) in the right ES-L4 (from 83% to 67%). The VEMP area (Table 1) did not significantly decrease in the ES-L4 ($F(1,5)=-0.4$, $p=0.7$ right side, and $F(1,5)=-0.9$, $p=0.4$ left side). By contrast, the response to GVS completed disappeared in the gastrocnemius (from 100% of the subjects to 0% in the left gastrocnemius, $Z= 3.5$, $p<0.001$; and from 67% to 0% in the right gastrocnemius, $Z=2.4$, $p<0.05$) with a reduction in the VEMP area close to null in the gastrocnemius ($F(1,5)=3.8$, $p<0.05$ right side, and $F(1,5)=2.7$, $p<0.05$ left side). Altogether, the harness condition reproduced the same effects as those observed in micro-gravity with a stable proportion of subjects expressing axial VEMPs in the upright standing and harness conditions despite the gating of VEMPs in the gastrocnemius.

DISCUSSION

We challenged the persistence of vestibular evoked myogenic potentials (VEMPs) in back muscles and leg muscle to determine how these muscles can be experimentally modulated. Our results strongly suggest that a differentiated control mechanism exists between axial and appendicular muscles when an imbalance is detected by vestibular inputs.

463 The finding that with null gravitational information from the otolithic system (microgravity),
464 we still observed persistent GVS elicited responses in axial muscles is particularly interesting.

465

466 **Functional responses to GVS in upright standing and phase-dependent effects during gait**

467 The significant postural changes induced by GVS in upright standing (Fig. 1) and the
468 mirror response observed when the anodal stimulation was delivered either to the right or
469 left mastoid process (Fig. 3B & 4) validate the reliability of the stimulation protocol. This
470 reliability was also confirmed under the various gravity conditions with a significant increase
471 in the kinematic changes when the postural demand was increased (Fig. 5CII).

472 During gait, the analysis of the lateral displacements following GVS indicates a global
473 translation of the trunk to the anode side, i.e., the side opposite to the detected displacement,
474 which is consistent with previous studies (Ali et al., 2003) and similar to the deviations
475 observed with prolonged GVS (Fitzpatrick et al., 1999; Bent et al, 2000). The higher magnitude
476 displacements in the upper parts of the body and the deviation of the swing foot in the reverse
477 direction correspond to a body rotation in roll (Day et al., 1997; For a review, see Fitzpatrick
478 and Day, 2004; Son et al., 2008) with a rotational axis located at the coxo-femoral articulation.
479 During gait, VEMPs in the gastrocnemius were only recorded in the loaded leg, and the muscle
480 discharge is increased when the loaded leg is ipsilateral to the anode, but the muscle activity
481 is decreased with the contralateral anode. Similar phase effects were observed in the tibialis
482 anterior (but with antagonistic action on the TA and GL; not presented). The phase-
483 dependency reported here is consistent with the phase effects observed in gait kinematic
484 (Bent et al., 2004; Dakin et al., 2013) and lower limb electromyography using stochastic GVS
485 (Blouin et al., 2011) and the role of ground contact forces during unloaded gait (Ivanenko et
486 al., 2002; Sylos-Labini et al., 2014). This modulation of gastrocnemius responses to GVS
487 contrasts with the functional inflexibility of the ES-L4 muscle responses that remained
488 constant along the gait cycle (Fig. 3).

489

490 **Differential gating of vestibular evoked myogenic potentials**

491 One of our most striking observations is the clear dichotomy between the functioning
492 of the vestibulo-spinal pathways impinging on either the axial or leg motoneurons. We
493 observed a systematic suppression of VEMPs in the gastrocnemius when the force output did
494 not contribute to the maintenance of body balance. This finding was observed (1) during the
495 swing phase of the cycle during walking; (2) with head contact in the upright standing position;
496 (3) in the absence of gravity to counteract; and (4) while the body was supported by a hip
497 harness.

498 Experiments performed in modified gravity environments may help provide important
499 insight into the sensorimotor integration of vestibular inputs and confirm that axial and leg
500 muscles are differentially controlled. In microgravity, we reported a complete disappearance
501 of VEMPs in the gastrocnemius muscle, in contrast to a major increase in the VEMP amplitudes
502 in hypergravity to match the increasing destabilizing external forces. This VEMP deletion was
503 not only observed during free-floating but also when cutaneous and proprioceptive feedback
504 was provided through the ankle under the foot-strapped condition. Similarly, when the
505 subjects were sustained in the upright position in a harness (Fig. 6B), i.e., a position similar to
506 that adopted in the free-floating condition but with otolithic and proprioceptive knowledge
507 of the context of normogravity, or stood upright with slight head contact (Fig. 4C), we still
508 observed the deletion of VEMPs in the gastrocnemius. Therefore, the modulation of VEMPs
509 in lower limbs is likely more related to body contact and support from the surroundings than
510 to the postural attitude as observed in seated subjects (Britton et al., 1993; Day et al., 1997).
511 The large decrease in the antero-posterior displacement of the center of pressure observed
512 here (Fig. 4A), which has also been reported by other scholars (Britton et al., 1993; Fitzpatrick
513 et al., 1994), has been interpreted as a gating of the vestibular input on the balance control,
514 which could be dependent on the congruence of sensory feedback (visual, proprioceptive, and
515 vestibular; Luu et al., 2012). Interestingly, when the subjects stood upright with slight head
516 contact, a decrease was observed in the frontward and backward sway (both GVS polarities),
517 demonstrating that this finding could not be explained only by the mechanical impossibility of
518 sway against the forehead contact.

519 In contrast to the above discussion regarding the suppression of GVS-induced activity
520 in the gastrocnemius muscle, a major result of the present study is the persistent responses
521 in ES-L4 following GVS under all tested conditions. This persistence of ES-L4 VEMPs is

522 particularly intriguing under the free-floating conditions in which the subjects were unplugged
523 from their surroundings with no functional requirement of postural adjustments (no
524 constraint of gravity on posture or balance and no visual frame of reference) without any body
525 effectors available for support. The vestibulo-spinal responses observed in our experiments
526 likely originated from the stimulation of the neuroepithelia of the cristae (in the ampula of the
527 semi-circular canals), which was accompanied by concomitant stimulation of the maculae
528 (Fitzpatrick et al., 2004). Thus, in microgravity, the otolithic signal was null, informing of the
529 lack of gravity, and likely became incongruent when GVS occurred. In this context, the
530 persistence of the axial response strongly suggests that the otolithic signal did not interfere
531 with the integration of the semi-circular afferents. Together with the very short latency of the
532 ES VEMP (47 ms versus 89 ms in the gastrocnemius in normal standing), the response of the
533 axial muscles to vestibular inputs is more direct and robust and cannot be modulated by the
534 context.

535 This dichotomy between the axial and leg responses to GVS could have functional
536 significance considering the involvement of the trunk and ankle muscles in postural control.
537 The interrelations between the body and its surrounding can be approached from the
538 following two references values serving to control erect posture (Massion et al, 1998;
539 Ivanenko and Gurfinkel, 2018) that can be dissociated in microgravity: one 'geometrical' value
540 relative to the orientation of the body segment ("posture") and one 'kinetic' value related to
541 the distribution of the body mass with respect to the support area ("equilibrium"). Space flight
542 experiments (Clément et al., 1984; 1988) have shown that the body schema, which is an
543 internal representation of the body geometry, was surprisingly stable in microgravity,
544 although extensive changes were observed in the labyrinthine and proprioceptive inputs. It
545 can be hypothesized that axial VEMPs persisting in microgravity may originate from a process
546 that supports the stability of the body schema. By contrast, Mouchnino et al. (1996) showed
547 that the postural adjustment observed at the ankle during voluntary leg raising disappears in
548 microgravity with an abolition of the shift of the center of mass (CM) toward the supporting
549 limb. The variable VEMP we observed at the lower limb muscles could be related to the kinetic
550 requirement of maintaining the center of pressure inside the polygon of sustentation, which
551 is a constraint that disappears with the loss of gravity.

552

553 **Origin of the gating of VEMPs in the lower limb**

554 Even if the exact mechanisms by which gating may occur remain unclear likely because
555 multiple processes may be involved, it is a ubiquitous property of most vestibular responses.
556 For example, the vestibulo-ocular reflex (VOR) can be suppressed during voluntary gaze
557 behavior directed toward a visual target (Laurutis and Robinson, 1986; Pelisson and Prablanc,
558 1986). Similarly, the vestibulocolic reflex, which stabilizes the head in space by generating a
559 command to move the head in the opposite direction (Colebatch et al., 1994), can be
560 suppressed by voluntary head motion (Ezure and Sasaki, 1978; Goldberg and Cullen, 2011).

561 Regarding the suppression of VEMPs in the leg muscles, several mechanisms can be
562 suggested to explain the gating. If during free floating, the persistence of VEMPs in the lower-
563 limb muscles can be considered behaviorally irrelevant to maintaining balance, under the
564 foot-strapped condition, the VEMPS also disappeared although there was direct active
565 involvement of the ankle muscles. This finding suggests that gating is partially based on the
566 detection of a gravitational field either by the otolithic system or extravestibular somatic
567 receptors (Mittelstaedt, 1992). However, under the harness or head-contact conditions in
568 normogravity, the detection of a downward gravitational field by upright subjects was not
569 sufficient to elicit VEMPs in the gastrocnemius. Altogether, these results suggest that the
570 incongruence among the vestibular, proprioceptive and cutaneous inputs abolishes the
571 VEMPs in the lower limb muscles. The vestibular responses in the legs could be evoked only
572 when all features of a nominal terrestrial postural task are met as follows: gravity must be
573 detected, segment must be involved in the postural task, and sensorial cues must be
574 congruent.

575 In their detailed study investigating the effects of vestibular stimulation during
576 walking, Iles and colleagues (2007) reported that gating occurred in the soleus muscle during
577 gait with similar phase-dependency as that observed in the present study, and these authors
578 proposed that gating may occur through interactions with the central pattern generator at the
579 lumbar level. Alternately, these authors also posed the hypothesis that gating could occur at
580 the level of the brain stem. However, if this was the case, the axial and lumbar motoneuron
581 responses could be affected in the same way since it has been recently shown in rodents that

582 the two motoneuronal populations are similarly targeted by the lateral vestibulospinal tract
583 (LVST; Kasumacic et al., 2010).

584

585 **Phylogenetic blueprint of vestibulo-spinal sensorimotor organization**

586 The shift from swimming to upright bipedal locomotion is an outstanding feature of
587 the evolutionary process, and the question of whether any of the underlying neural circuitries
588 and mechanisms responsible for locomotor output production have been preserved during
589 this transitional process remains unanswered. Several studies have previously suggested that
590 during this evolutionary transition, the core features of the spinal cord circuitry of most
591 primitive vertebrates, such as lamprey, have been conserved both in rats (Falgairolle et al.,
592 2007; Beliez et al., 2015) and humans (de Sèze et al., 2008; Ceccato et al., 2009 ; for a review,
593 see Falgairolle et al., 2006; Falgairolle et al., 2013). Nevertheless, the switch to terrestrial
594 modes of locomotion and the emergence of limbs have necessarily increased the complexity
595 of postural and locomotor control mechanisms, and currently, the trunk musculature is
596 required for activity in strict coordination with limb movements during locomotion (Combes
597 et al., 2004). Despite this more complex mode of coordination, the spinal networks
598 responsible for trunk activity, even during bipedal walking, are still based on a bilaterally
599 distributed spinal chain of oscillators as observed in lamprey (Grillner et al., 1995; Grillner and
600 Wallen, 2002) but with the addition of limb CPG circuitry and associated interconnecting
601 pathways. Using an amphibian model, several studies have explored the way in which during
602 metamorphosis, locomotor networks and output transition from a purely trunk CPG (in the
603 tadpole) to a limb CPG (Beyeler et al., 2008; for a review, see Sillar et al., 2008). Therefore, the
604 extent to which the observations in the present study are related to comparable traces of
605 primitive vestibulo-spinal pathways that could have been preserved in mammals is unclear.

606 Two main features of vestibulo-spinal responses may be highlighted in this context.
607 First, the latencies of the vestibulo-spinal responses are on average twice longer in the
608 gastrocnemius compared to those in the ES-L4 muscles (Table 2). Based on the typical
609 conduction velocities reported in several studies (60 and 90 m/s for the lateral and medial
610 vestibulo-spinal tract, respectively; Wilson et al., 1967; Akaike et al., 1973b; Akaike et al.,
611 1973a), the theoretical delays required to recording the onset of muscle responses can be

612 calculated. Assuming a 1 m length (from the head to the lower back) and a minimal conduction
613 velocity of 60 m/s, including several synaptic delays, the vestibular inputs could elicit a
614 response in approximately 30 ms in the ES-L4. In the gastrocnemius, this delay could be
615 increased by up to 40 ms by adding the axonal route (approximately 1 m at 100 m/s) from the
616 lumbar spinal cord to the muscle. Therefore, the dramatic differences between the latencies
617 recorded in the ES-L4 and gastrocnemius cannot be explained by the sole differences in the
618 target distances, suggesting that different pathways and/or mechanisms exist. However, it is
619 unlikely that the differential gating operated on ES-L4 and gastrocnemius is attributable to the
620 involvement of different pathways since the vestibular inputs to lumbar motoneurons are
621 derived solely from the LVST in adult mammals (Wilson and Yoshida, 1969), and a recent
622 studies (Graf, 2007 ; Kasumacic et al., 2010) demonstrated that only LVST establishes
623 connections with both ipsilateral and contralateral thoracic motoneurons. As mentioned
624 above, the possibility has been raised (Iles et al., 2007) that gating could occur at the spinal
625 level. The emergent complexity of the circuitry organization required for hindlimb control
626 could suggest that more complex local circuit interactions occur at the lumbar level. Recent
627 studies have highlighted a major difference in the organization of mammalian neuronal
628 circuits involved in postural and locomotor activities between the axial and lumbar level
629 (Falgairolle et al., 2007; Beliez et al., 2015). Specifically, it has been found that neuronal
630 activity propagates faster in the thoracic segments and that a strong slowdown occurs from
631 the lumbar segments (Cazalets, 2005 ; Falgairolle and Cazalets, 2007). It has been suggested
632 that as lumbar segments are intimately involved in hindlimb control, more complex local
633 circuit interactions could slow the propagation through this region. The same vestibulo-spinal
634 signal processing could occur at the lumbar level, which could explain the longer delay and
635 serve as a basis for the sensory gating of vestibular inputs.

636 According to the phylogenetic perspective, the vestibular system has been present
637 very early in most primitive vertebrates in a definite form sharing many common
638 characteristics with the vestibular system in mammals (Kasumyan, 2004). This high degree of
639 early development is likely related to the challenging situation faced by fishes in their three-
640 dimensional permanently moving natural surroundings in which the sense of equilibrium plays
641 an extremely important role in orientation, especially when vision is not involved. Detailed
642 studies investigating the vestibular control of swimming in lamprey have characterized the

643 commands sent to the spinal cord through reticulospinal neurons (Deliagina et al., 1992 ;
644 Deliagina et al., 2006). This ancestral demand for highly efficient vestibular control could be
645 the source of the strong hard-wired vestibulo-spinal inputs to axial motoneurons with a lower
646 degree of flexibility than late emerging limb motoneurons.

647

648 **CONCLUSION**

649 The results of the studies presented here are all suggestive of the differentiated
650 governance of axial and appendicular segments when body rotation is detected by vestibular
651 inputs. The persistent characteristics of the myogenic adjustments observed in the trunk even
652 in absence of gravity suggest the presence of inflexible reflex wiring functionally efficient to
653 maintain posture. However, the behaviors observed in the experiments during the transient
654 microgravity episodes should be more related to the unconstrained expression of the neural
655 circuitry adapted to terrestrial gravity than the response of adaptable processes to an unusual
656 gravitational field. Multiple testing of postural behaviors in variable gravitational fields (lunar,
657 Martian, etc.) could provide an opportunity to assess the level of the adaptability of these
658 sensorimotor processes in new spatial contexts.

659

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Figure legends

Figure 1. **Representative kinetic and EMG recordings during GVS-induced perturbations**. Recordings were performed in one subject with the head turned, vision occluded, and anode on the posterior mastoid. A, Backward displacement of the center of pressure. B, Electromyograms from (right) the erector spinae at the T7 and L4 levels, gastrocnemius and tibialis anterior.

Figure 2. **Rostro-caudal gradient of GVS-induced perturbation amplitudes during gait**. A, Lateral displacements of the head, scapular girdles, pelvian girdles, and feet following GVS. Traces presenting data averaged across all subjects (N=11) with SD. The loaded stance foot following GVS was deviated during the following step, resulting in longer latencies than the swing foot. B, Magnitude of lateral displacements at various segmental levels. There was a decreasing gradient in the displacement magnitude from the head to the pelvic girdle. The swing foot was deviated in the direction opposite to the upper part of the body.

Figure 3. **Phase-dependency of GVS-induced perturbations**. A, Averaged rectified EMG of the right and left erector spinae (L4) and gastrocnemius during walking. Two successive normalized cycles (0-100%) are presented. ES muscles present bilateral double burst during each locomotor cycle, and each burst occurred close to the double support phase. Right and left gastrocnemius activity alternated with the burst occurring at the end of the stance period. B, The activity of the ES muscles was increased by GVS with the anode ipsilateral to the muscle and decreased with the anode contralateral to the muscle regardless of the phase of the cycle at which GVS was delivered. By contrast, the gastrocnemius activity was enhanced by the contralateral stimulation and decreased by the ipsilateral stimulation during the stance phase only and not during the swing phase, during which no GVS effect was observed.

801 Figure 4. **Maintenance of vestibulo-spinal reflex in axial muscles in upright standing subjects**.
802 A, During freestanding (left panel), the anteroposterior body sway was dependent on the GVS
803 polarity (positive value = frontward COP displacement). The body sway was reduced close to
804 null when the head slightly touched a support (tight panel, head contact). COP traces were
805 averaged across all subjects (N=9). B, The medium latency response to GVS in the ES muscles
806 was dependent on the GVS polarity and persisted in the absence (left panel) or presence (right
807 panel) of a head contact. C, The medium latency response to GVS in the gastrocnemius muscle
808 was dependent on the GVS polarity and virtually abolished in the presence (right panel) of a
809 head contact.

810 Figure 5. **Experimental conditions during parabolic flight.** A, Recordings from the plane
811 altimeter (dashed line) and the accelerometer (black line, axis perpendicular to the plane
812 ground) during one parabola. Each microgravity period (22 s) was flanked by two hypergravity
813 periods (20 s), and the parabolas were separated by a steady flight period (>90 s). B, The
814 subjects stayed upright with their vision occluded and head turned. The distended tether
815 prevented the extreme excursion of the subject in microgravity. C, Displacements of the upper
816 parts of the body following GVS with the anode on the posterior mastoid in (I) the lateral,
817 vertical and sagittal plane of the head during steady flight and (II) the sagittal plane during
818 steady flight (continuous line), microgravity (dotted line) and hyper gravity (dashed line) for
819 the head, the scapular and the pelvian girdle.

820 Figure 6. **Maintenance of vestibulo-spinal reflexes in axial muscles with decreasing postural**
821 **constraints.** A, During upright standing, VEMPs (rectified EMG synchronized to the GVS start
822 and averaged across the subjects) were elected by GVS (anode on the posterior mastoid
823 process) from the erector-spinae L4 (left panels) and gastrocnemius (right panels) muscles. In

824 microgravity, VEMPs were no longer observed in the gastrocnemius but were still present in
825 the erector spinae. B, Under laboratory conditions, the VEMPs elicited by GVS from the
826 erector-spinae L4 (left panels) and gastrocnemius (right panels) muscles were observed during
827 freestanding in both the ES and gastrocnemius muscle but disappeared from the
828 gastrocnemius muscles when the subjects were suspended upright in a harness.

829 Table 1. VEMP area under various conditions. Under the static conditions (modified gravity,
830 harness, and head contact), the areas were normalized by the VEMPs observed under the
831 adjoining control condition (i.e., 100% = VEMP area in free upright standing in normogravity).
832 For the VEMPs during gait, the areas are expressed as a percent of the median activity
833 measured during the gait cycles without GVS. Values represent the means ± SD.

834 Table 2. Averaged latencies of VEMPs measured in all experiments. Non-applicable (*"NA"*) is
835 assigned when VEMPs were not observed under the corresponding condition. Values
836 represent the means ± SD.

837

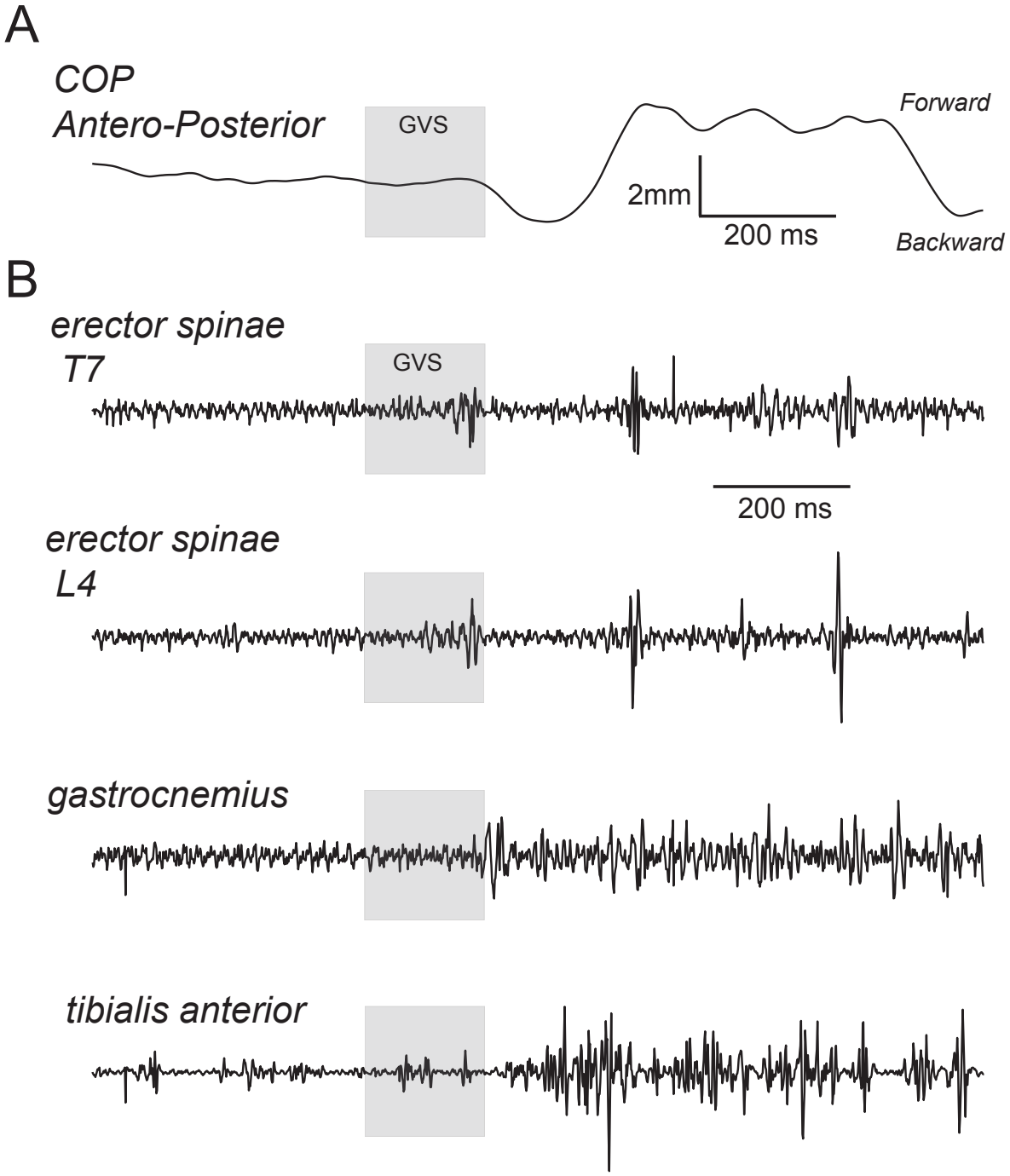

A
COP
Antero-Posterior
GVS
Forward
2mm
200 ms
Backward
B
erector spinae
T7
GVS
200 ms
erector spinae
L4
gastrocnemius
tibialis anterior

Figure 1

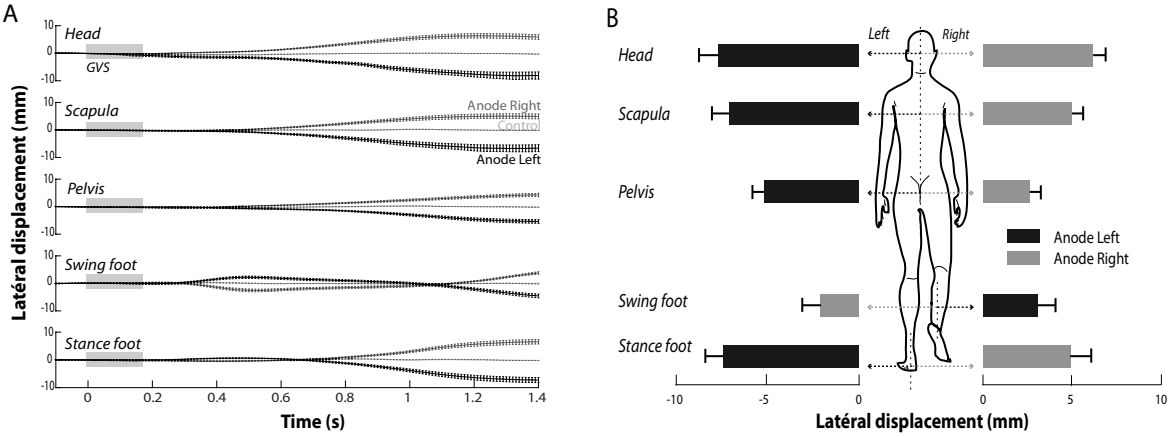


Figure 2

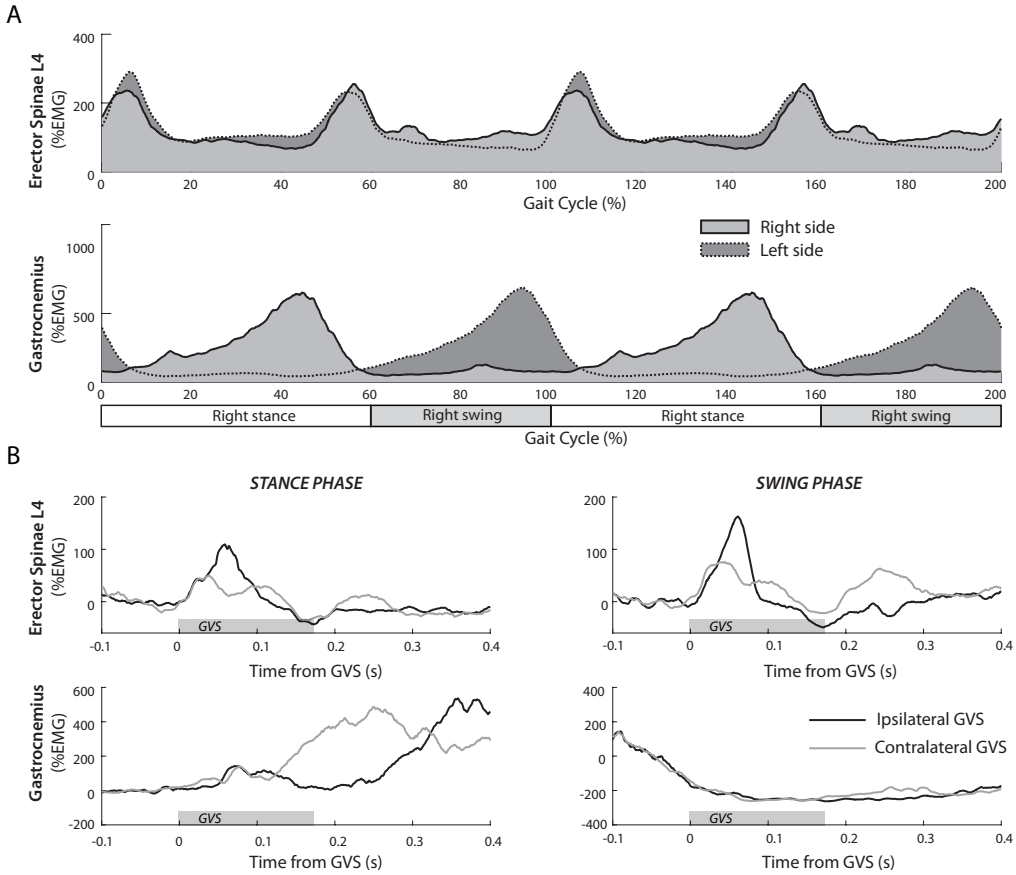


Figure 3

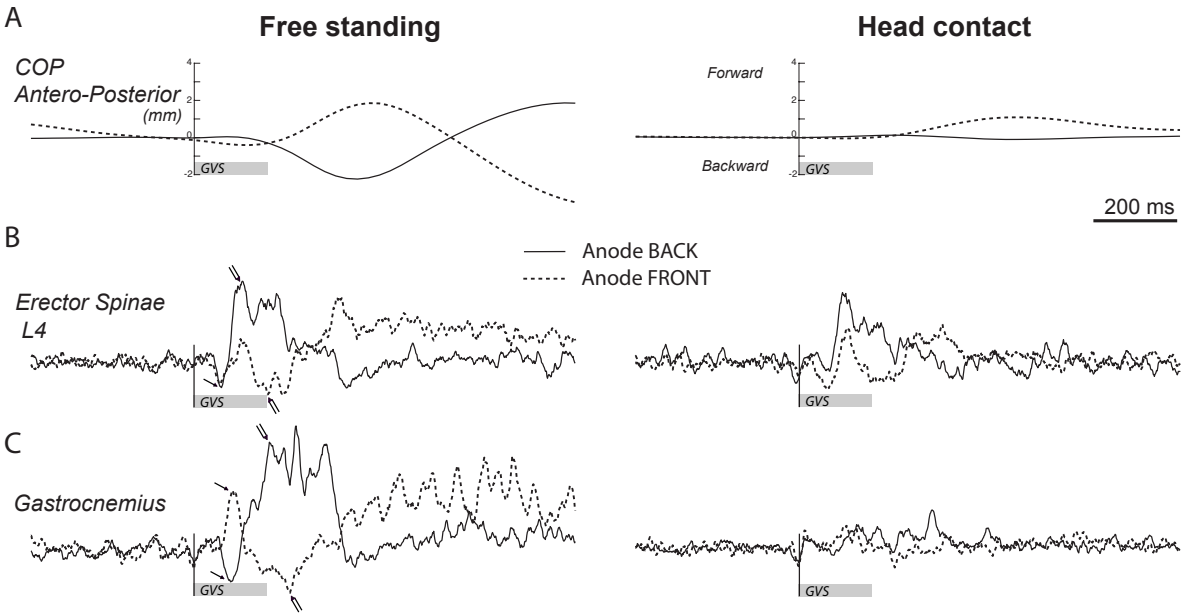

A
Free standing
Head contact
COP
Antero-Posterior
(mm)
Forward
Backward
GVS
200 ms
B
Anode BACK
Anode FRONT
Erector Spinae
L4
C
Gastrocnemius

Figure 4

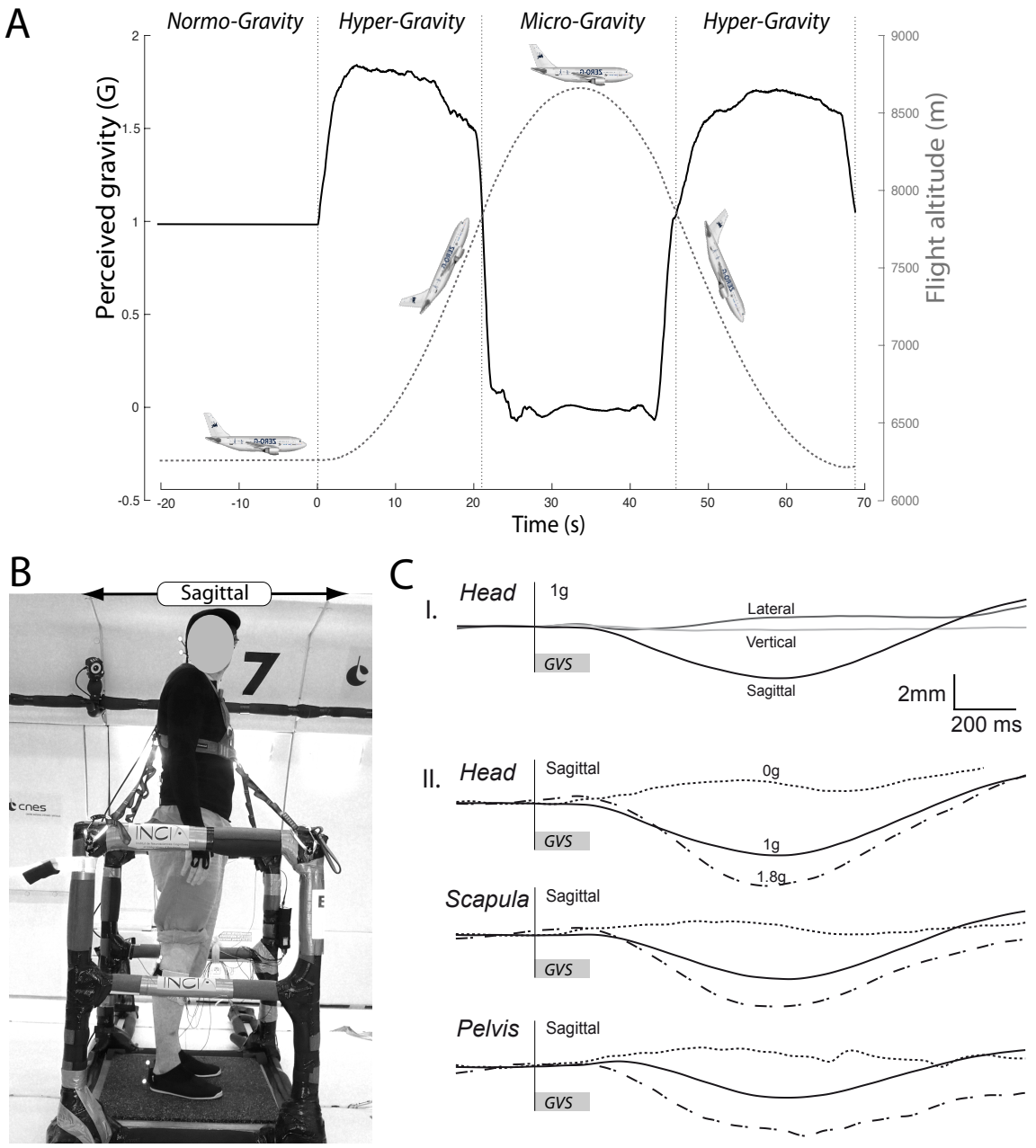
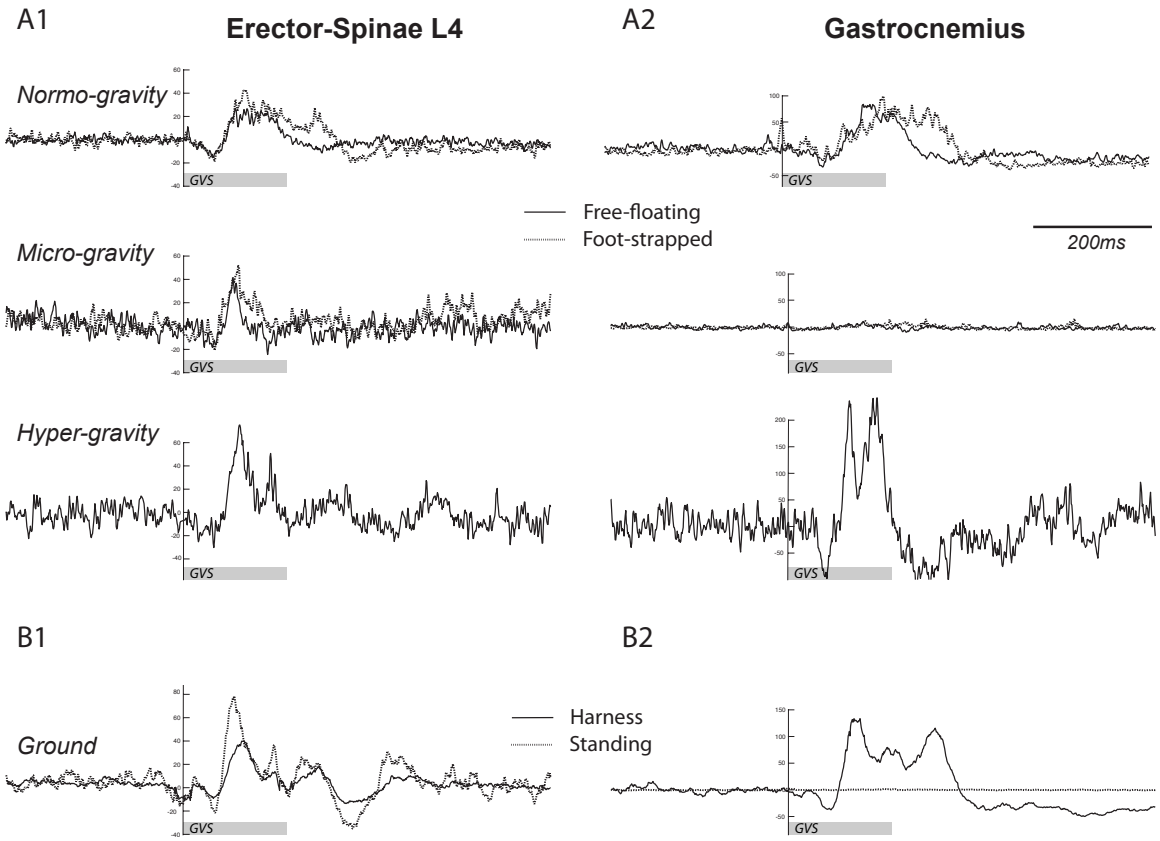

A
Normo-Gravity
Hyper-Gravity
Micro-Gravity
Hyper-Gravity
Perceived gravity (G)
Flight altitude (m)
Time (s)
B
Sagittal
C
I.
Head
1g
Lateral
Vertical
Sagittal
GVS
2mm
200 ms
II.
Head
Sagittal
0g
1g
1.8g
Scapula
Pelvis

Figure 5



853

854 **Figure 6**

855

VEMPs areas (% of normal standing ± SD)	Parabolic Flights			Ground		VEMPs areas (% of no GVS ± SD)	Gait			
	Hypergravity	Microgravity		Harness	Standing with Head Contact		Load Ipsilateral		Load Controlateral	
	Free floating	Free floating	Foot strap				Anode Ipsi	Anode Contra	Anode Ipsi	Anode Contra
ES-L4	232 ±123	105 ±114	82 ±42	138 ±117	97 ±102	**ES-L4**	106 ±37	83 ±33	119 ±35	92 ±22
Gastrocnemius	251 ±157	-1 ±19	20 ±30	2 ±6	28 ±15	**Gastrocnemius**	96 ±55	201 ±99	48 ±38	55 ±33

Table 1

VEMP Latencies (ms)		Condition	ES-L4	Gastrocnemius
Parabolic Flights	**Normogravity**	*Free floating*	72 ±13	108 ±15
		Foot strap	61 ±16	101 ±20
	Hypergravity	*Free floating*	62 ±7	84 ±11
	Microgravity	*Free floating*	57 ±6	*NA*
		Foot strap	61 ±10	*NA*
Harness		*Sustained*	65 ±14	*NA*
Standing		*Free standing*	47 ±35	89 ±16
		Head Contact	51 ±22	*NA*
Gait		*Appui Ipsi*	41 ±10	136 ±14
		Appui Contro	42 ±11	144 ±20
			ES-T7	**Tibialis Anterior**
		Appui Ipsi	36 ±7	161 ±27
		Appui Contro	33 ±8	185 ±28
KINEMATIC		**Body part**	**Latencies (ms)**	
Gait		*Head*	203 ±135	
		Scapular girdle	333 ±105	
		Pelvic girdle	343 ±113	
		Swing foot	214 ±47	
		Stance foot	533 ±127	

Table 2