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Research Paper

Preparatory postural adjustments during gait initiation in healthy younger and older adults: Neurophysiological and biomechanical aspects



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ABSTRACT

The most postural adjustments studies have focused on electromyographic and biomechanical events to identify the mechanisms involved in falling, whereas direct recording of central processor system could be an optimal approach to provide new insights into this field. Therefore, the purpose of this study was to determine neurophysiological and biomechanical aspects of the preparatory postural adjustments during gait initiation (GI) in healthy younger and older adults. 16 healthy younger and 15 healthy older adults participated in the study. Stimuli of warning and response were played with an inter-stimulus interval of 2 s and subjects were instructed to begin forward stepping with the dominant limb in response to response stimulus. The contingent negative variation (CNV), onset time of electromyographic activity in leading limb muscles and center of pressure (COP) trajectory in the anticipatory phase of GI were measured. Results revealed the peak time of CNV and mean amplitude of Late CNV were earlier and smaller in older group, respectively. However, peak amplitude of CNV did not differ significantly between groups. Moreover, the older group exhibited a delayed onset activity of gluteus medius and tibialis anterior muscles as well as a slower velocity and lesser backward displacement of COP trajectories in comparison with the younger group. Additionally, there were strong relationships between neurophysiological and biomechanical parameters. Findings suggest the age-related changes in the brain activities and preparatory postural adjustments during GI which could be valuable in designing assessment and prevention approaches for falls in this group of people. © 2015 Elsevier B.V. All rights reserved.

1. Introduction

Falls are a critical problem facing adults over 65 years old and often lead to physical, social, and psychological consequences for this group (Silsupadol et al., 2006). Older subjects frequently experience falls during the transitional phases of gait like initiation, termination or turning. Thus, initiation of gait is commonly a precarious task among them

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(Polcyn et al., 1998). Indeed, gait initiation (GI) is a voluntary transition from a state of relatively stable to a state of continuously unstable (Hass et al., 2004; Uemura et al., 2012b), which is characterized by a preparatory phase that precedes the initiation of stepping and an execution phase that propels the body in the intended direction (Rogers et al., 2011; Rosin et al., 1997). In the preparatory phase, the central nervous system (CNS) elicits motor responses to prepare the body for movement. This process, named anticipatory postural adjustments (APAs), involves a sequence of muscle activities allowing the center of pressure (COP) to move backwards, prior to the onset of the first step (Rogers et al., 2011). Indeed, the deactivation of the gastrosoleus that accompanies the activation of the tibialis anterior (TA), is responsible for the backward displacement of the COP, generating a controlled forward displacement of the center of mass (COM) over a changing base of support (Ko et al., 2011). In sum, these APAs diminish the forces applied to the swing limb and are requisite for forward progression (Cau et al.,

Some researchers believe the anticipatory manner of the TA is a prerequisite of preparatory adjustments during GI disturbed following ageing and other disabilities, thus consider it as an optimal index for scaling APAs (Isaias et al., 2014). Moreover, some studies have shown the backward shift of COP during GI is a sensitive scale for falls and balance dysfunctions that additionally has a substantial correlation with the amplitude of the TA activity (Crenna and Frigo, 1991; Uemura et al., 2012c). In these studies, elderly fallers have indicated a smaller and slower backward displacement of COP than non-fallers (Uemura et al., 2012a).

The Study of APAs could offer valuable insights into the role of CNS in the control of posture because those appear in advance of movement, therefore it is suggested that central processor system pre-programs the APAs prior to movement onset (Jacobs et al., 2010). Specific areas of the central nervous system such as the prefrontal cortex, primary motor area, premotor area, supplementary motor area, and basal ganglia is engaged in generating APAs (Fujiwara et al., 2012; Jacobs et al., 2010; Massion, 1992). Additionally, the prominent role of these regions in motor programming and planning has also been well known (Groenewegen, 2003; Massion, 1992; Sadato et al., 1997). Thus, a delayed and reduced APA activity in elderly subjects indirectly indicates the alterations of the central processor system.

Most postural adjustments studies have focused on electromyographic and biomechanical events to scale the systems of feed forward control and to identify the mechanisms involved in falling, whereas direct recording of central processor system could be an optimal approach to provide new insights into the association of delayed and reduced APAs, evidenced by biomechanical and electromyographic events, with neurophysiological changes of the central system.

The central system activity prior to the voluntary movements can be investigated using event-related brain potentials (ERPs)(Fujiwara, 2013). The contingent negative variation (CNV) is an ERP signal characterized by a slow negative shift of EEG amplitude in the preparatory period between the stimuli of warning and response (Tomita et al., 2012). The CNV contains two main components of 'Early' and 'late', such

that late component is related to the motor preparation process, anticipatory attention and cognitive processes (Fujiwara et al., 2012; Leuthold and Jentzsch, 2009). Several regions of the brain, including the prefrontal cortex, primary motor area, premotor area, supplementary motor area, somatosensory cortex, and basal ganglia, contribute to the production of late CNV (Fujiwara et al., 2012). As mentioned earlier, most of these areas are also involved in the generation of APAs as well as motor programming and planning, impelling the presence of relationship between late CNV, APAs, programming, and planning. In support of this neurophysiological overlapping of the late CNV and the APAs, some studies have shown that the timing of postural muscle activities is related to alterations in CNV (Shen et al., 2009).

Although physiological aging is a process that is commonly accompanied by modulation of brain structure and many neurological disorders, little is known about the neurophysiological changes of the brain in this phase of life (Golob et al., 2005). Earlier CNV studies with finger flexion and key pressing tasks have implied the negative peaks of late CNV are reduced or even absent in the older group (Golob et al., 2005; Michalewski et al., 1980). In addition, a relationship between the age-related changes in the function of the frontal lobe and postural adaptation in elderly subjects has been reported (Fujiwara et al., 2012). Few studies have investigated the brain potentials during stepping (do Nascimento et al., 2005; Vidailhet et al., 1995; Yazawa et al., 1997). However, available evidence about the association between brain potentials and postural preparation prior to GI for older adults has not been sufficiently discussed. Recording brain activities prior to GI may give additional insight into how the cerebral cortex of older adults contributes to postural preparation, as compared to younger adults. Accordingly, identifying changes of the cerebral cortex could be valuable in designing assessment and prevention approaches for falls in this group of people. Therefore, the purpose of this study was to identify the age-related changes in the brain activities and preparatory postural adjustments during GI.

2. Results

2.1. EEG findings

The results of EEG analysis can be seen in Table 1. These findings revealed the peak amplitude of CNV did not differ significantly between groups. However, the peak time of CNV was considerably different between both groups at all electrodes, such that in older adults, signals would reach the peak earlier in comparison with younger adults (i.e. The CNV peak of younger group was closer to S2 than it in older group). Moreover, the mean amplitude of Late CNV was reduced in older relative to younger adults at all electrodes; however, this difference was significant only for Fz.

2.2. EMG findings

Findings of EMG analysis are presented in Table 2. In this study, the older group exhibited a delayed onset activity of

Table 1 – Measurement parameters of electroencephalographic analysis in the younger and older groups.

Parameter	Electrode	Subject				p Value	
		Younger		Older			
		Mean	SD	Mean	SD		
Peak	Fz	20.10	13.88	16.40	10.26	0.383	
amplitude	Cz	14.70	6.55	14.85	10.90	0.962	
of CNV [μv]	Pz	13.00	6.32	17.13	11.89	0.216	
Peak time of	Fz	412.22	279.18	778.95	479.85	0.010	
CNV [ms]	Cz	466.91	371.11	774.81	479.16	0.044	
	Pz	375.91	325.13	816.63	572.82	0.010*	
Mean	Fz	9.55	6.24	3.90	5.51	0.008*	
amplitude	Cz	7.27	3.75	5.73	6.82	0.419	
of Late CNV [μν]	Pz	7.80	4.52	5.19	5.97	0.157	

Abbreviations: CNV: contingent negative variation.

Table 2 – Measurement parameters of electromyographic analysis in the younger and older groups.

Parameter	Muscle	Subject				p Value
		Younger		Older		
		Mean	SD	Mean	SD	
Time to activity	GM	309.46	165.56	610.48	388.02	0.000*
onset [ms]	RF	481.97	134.90	578.81	216.49	0.129
	VM	517.17	143.86	619.91	205.86	0.064
	VL	537.45	181.10	647.24	277.39	0.079
	BF	583.32	184.86	502.12	286.36	0.157
	ST	639.42	153.58	607.34	239.30	0.175
	TA	266.80	105.88	327.96	213.59	0.049*
	GS	574.32	284.12	448.73	242.40	0.707

Abbreviations: GM: gluteus medius; RF: rectus femoris; VM: vastus medialis; VL: vastus lateralis; BF: biceps femoris; ST: semitendinosus; TA: tibialis anterior; GS: gastrocnemius medialis

gluteus medius (GM) and TA muscles in comparison with the younger group.

2.3. COP findings

It was found that the older group had slower velocity and lesser backward displacement of COP trajectories than the younger group. However, no significant difference was seen between the groups for other parameters (Table 3).

2.4. Linear relationships

Associations between the parameters of EEG, EMG and COP, quantified by Pearson's moment correlations, have presented in Table 4. However, it should be mentioned that only associations that had level of statistical significance less than 0.05 in each of groups, have presented. In younger group, the

Table 3 – Measurement parameters of center of pressure analysis in the younger and older groups.

Parameter	Subject				p Value
	Younger		Older		
	Mean	SD	Mean	SD	
Time to displacement onset [ms]	632.82	58.19	600.49	145.27	0.417
Displacement of COP (ML) [cm]	2.08	0.75	2.05	0.88	0.918
Displacement of COP (AP) [cm]	2.00	1.13	1.13	0.72	0.007*
Velocity of COP Displacement (ML) [cm/s]	7.92	2.96	9.59	4.28	0.162
Velocity of COP Displacement (AP) [cm/s]	8.22	3.89	5.82	3.34	0.043*

Abbreviations: COP: center of pressure; AP: Anteroposterior; ML: mediolateral

peak amplitude of CNV in Cz was significantly negatively associated with onset activity of the vastus medialis (VM) and semitendinosus (ST) muscles. Indeed, the more delayed onset of muscles was associated with smaller CNV amplitudes. Also, the peak time of CNV in Cz and Fz was correlated with onset activity of TA and vastus lateralis (VL) muscles, respectively. So that, the earlier onset of muscle activities was associated with the earlier peak time of CNV (i.e. later relative to S2). In addition, there was a strong, positive correlation between the peak time of CNV in CZ and Pz with the velocity and displacement of the COP in anteroposterior (AP) direction. In other words, the higher velocity and larger displacement of COP was related to the earlier peak time of CNV. However, in older group, only the larger displacement of the COP in mediolateral direction was associated with larger CNV amplitudes in Fz.

The associations between the EMG and COP parameters also investigated in this study (Fig. 1). In younger group, the more delayed onset of the GM and TA muscles were associated with lesser displacement of the COP in AP direction. The same trend was evident for the relationship between the onset of TA muscle and the velocity of COP Displacement in AP direction as well as the relationship between the onset of gastrocnemius (GS) muscle and the displacement of COP in ML direction in both groups.

3. Discussion

The results of current study demonstrated that postural preparation mechanisms prior to GI could be impaired in older adults. Also, the close correlation among the EMG and COP parameters with EEG parameters confirmed this notion that CNS governs the initiation of gait by pre-designed motor programs (Hass et al., 2004; Polcyn et al., 1998). The results exhibited the mean amplitude of Late CNV in Fz is reduced in

Significant difference between younger and older groups (independent T-test, p < 0.05).

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Table 4 – Linear relationships between measurement parameters of EEG and parameters of EMG and COP in younger a	nd
older groups.	

	Touriger addits rearson 57 (p varae)	Older adults Pearson's r (p-value)
Velocity of COP Displacement (ML)	0.001 (0.998)	0.670 (0.012)
Time to activity onset of VL	-0.523 (0.046)	-0.064 (0.843)
Time to activity onset of VM	-0.516 (0.049)	0.191 (0.533)
Time to activity onset of ST	-0.540 (0.038)	0.202 (0.509)
Time to activity onset of TA	-0.627 (0.012)	-0.084 (0.785)
Displacement of COP (AP)	0.643 (0.010)	-0.078 (0.801)
Velocity of COP Displacement (AP)	0.659 (0.008)	0.020 (0.947)
Displacement of COP (AP)	0.592 (0.020)	-0.052 (0.867)
Velocity of COP Displacement (AP)	0.625 (0.013)	0.040 (0.896)
	Time to activity onset of VL Time to activity onset of VM Time to activity onset of ST Time to activity onset of TA Displacement of COP (AP) Velocity of COP Displacement (AP) Displacement of COP (AP)	Time to activity onset of VL

Correlation is significant at the 0.05 level (2-tailed). r is Pearson product-moment correlation coefficient.

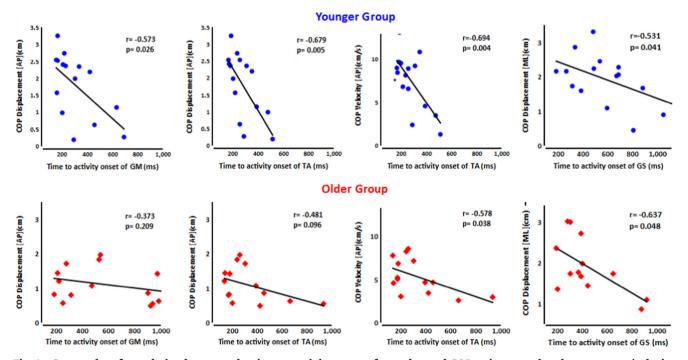


Fig. 1 – Scatterplot of correlation between the time to activity onset of muscles and COP trajectory related parameters (velocity and displacement of COP) in younger and older groups. Correlation is significant at the 0.05 level (2-tailed). r is Pearson product-moment correlation coefficient.

older relative to younger adults. Late CNV is related to the motor preparation process, anticipatory attention and cognitive processes (Fujiwara et al., 2012; Leuthold and Jentzsch, 2009). Therefore, consistent with earlier studies, it was appeared that motor preparation and cognitive processes are reduced in older people (Golob et al., 2005; Michalewski et al., 1980). Earlier CNV studies with finger flexion and key pressing tasks have indicated the negative peaks of late CNV are reduced or even absent in the older group (Golob et al., 2005; Michalewski et al., 1980). Reduced Late CNV could be due to deterioration of neural circuits generating the CNV, such as neural networks of supplementary motor area, premotor area and basal ganglia. It has been well known that these regions play a prominent role in preparing to initiate voluntary movements as well as in motor programming and planning of sequential movements (Groenewegen, 2003; Massion, 1992; Sadato et al., 1997). Indeed, the overall strategy

of movement is determined in motor planning which recruits and runs motor programs. The motor programs select a series of suitable subroutines for the intended movement and then designate a particular group of muscles to provide these subroutines and additionally regulate correct timing of muscle activation (Okada et al., 2011). In other words, the motor program defines the detailed characteristics of movement, including the temporal sequence of muscle activity, the duration of activity, and the force that each muscle generates (Leuthold and Jentzsch, 2009). Therefore, the coordinated movements depends on the accurate timing in the running and switching of motor programs. (Groenewegen, 2003). Accordingly, decrease and/or degeneration of neurons, dendrites and/or neural synapses as well as depletion of neurotransmitters (e.g. Dopamine within the basal ganglia) with advancing age could disturb the function of these regions, which in turn, may alter the activation of muscles, the same

thing observed in this study for timing of GM and TA muscles. In the present study, the older group exhibited a delayed onset activity of GM and TA muscles in comparison with the younger group. Subsequently, it might be concluded that the displacement of COP is also affected as a result of such alterations, as was observed in this study. Findings revealed that older group has slower velocity and lesser backward displacement of COP trajectories than the younger group. In anticipatory phase, the relaxation of GS and the contraction of TA create a dorsiflexion moment resulted in the backward displacement of the COP, and the relaxation of GM of the trailing limb and the contraction of GM of the leading limb move COP toward the leading limb (Park et al., 2009). Subsequently, separation of the center of mass and the center of pressure conduct the center of mass forwards and towards the trailing limb (Fiolkowski et al., 2002). So, a delayed onset activity of GM and TA muscles may partly explain the cause of such findings. This claim is further confirmed by results of correlation analysis, which reflected the more delayed onset of TA muscle is associated with the slower and lesser backward displacement of the COP. Interestingly, findings of correlation analysis of both groups implied that GS muscle might be engaged in moving COP not only in AP direction, but also in ML direction.

In addition, reduced Late CNV and attenuation of postural preparation could be attributed to decreased performance of somatosensory cortex in older people. In order to have an efficient motor planning, organization and integration of sensory inputs from the body as well as interpretation of information are the essential prerequisites (Ayres, 1972). Difficulty in sensory processing might lead to a poor motor planning for movement initiation and then an inefficient postural preparation.

As mentioned earlier, the results of this study displayed the mean amplitude of Late CNV of both groups is significantly different only over Fz electrode. This finding could be in line with this view that age-related brain changes are more prominent within the frontal lobes relative to other brain regions. In other words, the aging process might selectively deteriorate this region (Greenwood, 2000).

On the other hand, it was found that the peak time of CNV is considerably different between the groups at all electrodes, such that in older adults, signals would reach the peak earlier in comparison with younger adults. It has been proposed that the peak time of the CNV is relevant to the focal point of anticipatory attention (Maekawa et al., 2013). Thus, reduction of anticipation with aging process, and therefore the inability of older adults to predict the time of stimulus may be resulted the signals to reach a peak earlier. In this line, Tecce study has displayed the negative CNV rise gradually as the subject is confident about the time of the response stimulus, and rise quickly as the subject is uncertain about the time of response stimulus (Tecce, 1972). Moreover, in Fujiwara study, it was found as response stimulus is played later than expected time, such changes in CNV elicit (Fujiwara et al., 2011). Furthermore, study by Fujiwara et al. has exhibited as the balance difficulty increases, the CNV peak become larger and earlier. They suggested in difficult condition, a greater extent of attention is necessitated than the simple condition and postural preparation are triggered earlier (Fujiwara et al.,

2011). On the other hand, several researchers have reported that attentional demands of control posture is increased in older adults, even under relatively simple conditions (Woollacott and Shumway-Cook, 2002). Therefore, according to these views, it could be assumed that gait initiation is a more difficult task for older relative to younger people due to different attentional capacity. Thus, the peak CNV reaches peak earlier, to provide adequate time for postural preparation. However, the peak amplitude of CNV did not differ significantly between groups. It could be as a result of interaction between several factors which have different effects on the CNV amplitude. Although performing a more attention demanded task may necessitate the increased CNV amplitude in older group, age-related difficulties in inhibition of the processing of irrelevant or distracting information within the experimental context, conflict or reduce availability of resources that could attenuate the CNV amplitude (Schneider-Garces et al., 2010). Therefore, it seems the combined effects of these factors on the CNV amplitude causes the differences between groups to not be statistically significant.

Moreover, the studies have indicated the magnitude of APAs depends mostly on step velocity, and, in fewer amounts, to step length (Rocchi et al., 2006). On the other hand, the step velocity and length may be slower and shorter in the older subjects than in the younger subjects, respectively. Therefore, there is a possibility that the observed differences in CNV and APAs in the two groups be due to differences in voluntary movement features itself. Thus, to clarify this issue, it is suggested that further studies in which younger adults initiate step with same velocity and length as older adults are performed.

Also, the results of correlation analysis in younger group revealed the earlier and larger CNV peak amplitudes are associated with the earlier onset of muscles as well as the higher velocity and larger backward displacement of COP. However, in older group, only positive correlation of CNV amplitudes in Fz and the velocity of COP displacement in ML direction were observed. These strong associations of biomechanical and electromyographic events with neurophysiological changes of the central system further confirm that the CNV represents the anticipatory motor preparation prior to gait initiation in younger group. However, such associations were not observed within older group except for one case mentioned. The studies have reflected that the CNV may be related to the dopaminergic system (Schevernels et al., 2014) thus the earlier onset of muscle activities may be brought about by the greater excitability of neural circuits within the task-related cortex prior to the motor initiation. However, since in older adults balance between inhibitory and excitatory neural circuits is impaired and they are unable to selectively recruit neural networks (Schneider-Garces et al., 2010), therefore statistical correlation between parameters may not be clearly manifest.

4. Limitations

The major limitation of this study is that step velocity and length were not controlled to allow a behavior as natural as

Table 5 – Demographical and clinical characteristics.						
Parameter	Subject					
	Younger M:6)	(N=16; F:10,	Older (N=15; F:9, M:6)			
	Mean	SD	Mean	SD		
Age (years)	26.12	3.1	71.03	3.7		
Height (cm)	170.34	8.7	165.11	10.5		
Weight (kg)	56.60	2.6	69.40	3.5		
BBS (range 0-56)	56	0.0	54.3	1.1		
ABC (range 0-100)	100	0.0	83.55	10.9		
MMSE (range 0-30)	30	0.0	28.90	1.1		
TUG (s)	8.1	2.3	13.00	4.14		
HADS-D	4.3	2.8	5.5	2.6		

Abbreviations: BBS: Berg Balance Scale; TUG: Timed-Up & Go Test; ABC: Activities-specific Balance Confidence Scale; MMSE: Mini-Mental State Examination; HADS-D: Hospital Anxiety and Depression Scale-depression subscale; F: Female, M: Male

possible in both groups. However, as mentioned earlier, there is a possibility that differences in voluntary movement features could have influenced the effects of age on APAs mechanisms.

5. Experimental procedures

5.1. Subjects

16 healthy younger (6 males, 10 females; age, 26.12 ± 3.1 years) and 15 healthy older adults (6 males, 9 females; age, 71.03 ± 2.7 years) participated in the study (Table 5). Older subjects were screened for balance ability using the Berg Balance Scale (BBS), for functional mobility using the Timed-Up & Go Test (TUG), for balance confidence using the Activities-specific Balance Confidence Scale (ABC), for cognitive state using the Mini-Mental State Examination (MMSE) and for depression level using the Hospital Anxiety and Depression Scale- depression subscale (HADS-D).

The inclusion criteria for healthy older adults were the following: Age \geq 65 years old; BBS score > 40 that indicates subjects were in a low fall risk; Timed Up & Go score ≤ 20 seconds that indicates subjects were mostly independent; ABC score \geq 50% that indicates subjects were in a moderate to high level of physical functioning; MMSE score \geq 24 and HADS- depression subscale score \leq 7 that indicate subjects had no cognition and depression problem, respectively. Also, subjects had no severe cardiopulmonary disease, neurological disorder, musculoskeletal impairment or any history of falls in the prior 6 months. These cut off scores served to select a sample that consisted of healthy older adults who were in a generally good physically and mentally shape. Subjects were excluded from either group if had any dizziness, fatigue, vigorous physical activity or stress before testing. All Subjects signed an informed consent approved by the Institutional Ethics Committee of the Tehran University of Medical Sciences [No. 92.D.130.888].

5.2. Experimental protocol

The subjects stood barefoot and relaxed on the force platform, while eyes were open, both arms were hanged at the sides, feet were abducted at 10° and heels were separated mediolaterally by 6 cm (Uemura et al., 2012c) and weight was equally distributed. Subjects were requested to gaze at a fixation target placed about 3 m in front to minimize eye blinks and eye movements. To ensure that position of the feet was the identical across trials, an outline of each foot was drawn on the paper. A pair of auditory stimuli was played with an inter stimulus interval of 2 s through a loudspeaker placed 1 m behind the head of the subject. First stimulus (S1) and second stimulus (S2) were a warning and a response stimulus, respectively. The intensity, duration and frequency of both auditory stimuli were 60 dB, 100 ms and 2 kHz, respectively.

Subjects were instructed to focus on stimuli and begin forward stepping with the dominant limb as soon as possible in response to S2. Subjects were given no specific instructions regarding the velocity or the length of steps to allow a behavior as natural as possible.

Data were inspected after each trial to ensure that the subject did not begin stepping after the presentation of S1 or with the non dominant limb. Two or three experimental trials were performed before the main trials to familiarize the subjects with the test and overall, 20 trials for each subject were collected. All recording systems were synchronized in time and acquisition of them was triggered 2 s prior to the warning stimulus.

5.3. EEG recording

Brain activity was recorded using Ag–AgCl sintered ring electrodes and a 64 channel EEG system (Brain Quick System 98, Micromed, Mogliano Veneto, Italy) (A/D convertor: 32 bit, gain: $20~\mu\text{V/Div}$ and band-pass filter: 0.02~Hz low cut-off, 70~Hz high cut-off). Electrodes Fz, Cz and Pz were located on the scalp according to the International 10–20 system. These electrodes were referenced to a common reference electrode placed on the right mastoid process in a monopolar montage, as well as a ground electrode was attached to the Fpz. Moreover, four bipolar electrodes were used for the EOG recordings, two electrodes placed on the outer canthi of the eyes for monitoring horizontal eye movements, and two electrodes placed above and beneath the right eye for monitoring eye blinks (Tomita et al., 2012). All electrode impedances were kept below $5~\text{k}\Omega$. Signals were sampled at 256 Hz.

5.4. EMG recording

Surface electromyographic (EMG) activity of gluteus medius (GM), rectus femoris, vastus medialis (VM), vastus lateralis (VL), biceps femoris (BF), semitendinosus (ST), tibialis anterior (TA) and gastrocnemius medialis (GS) of the leading limb (limb which would take the first step in GI) was recorded using integral dry reusable electrodes (SX230, Biometrics Ltd, Gwent, UK) (Diameter: 10 mm, bipolar configuration and interelectrode distance: 20 mm) and an eight-channel EMG system (DataLog P3 × 8, Biometrics Ltd., Gwent, UK) (CMRR:

>96 dB at 60 Hz, input impedance>10¹² Ω , gain: 1000 and band-pass filter: 20 Hz low cut-off, 450 Hz high cut-off). After shaving and cleaning the skin with alcohol, electrodes were placed on the belly of each muscle in line with fiber direction, according to SENIAM guidelines (http://www.seniam.org), as well a ground electrode was attached to the subject's wrist. All electrode cables were tightly fixed to the skin to reduce

any movement artifacts. Signals were acquired at a sampling frequency of 1 KHz.

5.5. Force plate recording

Ground reaction forces and center of pressure trajectories were recorded by force platform (Bertec Corporation,

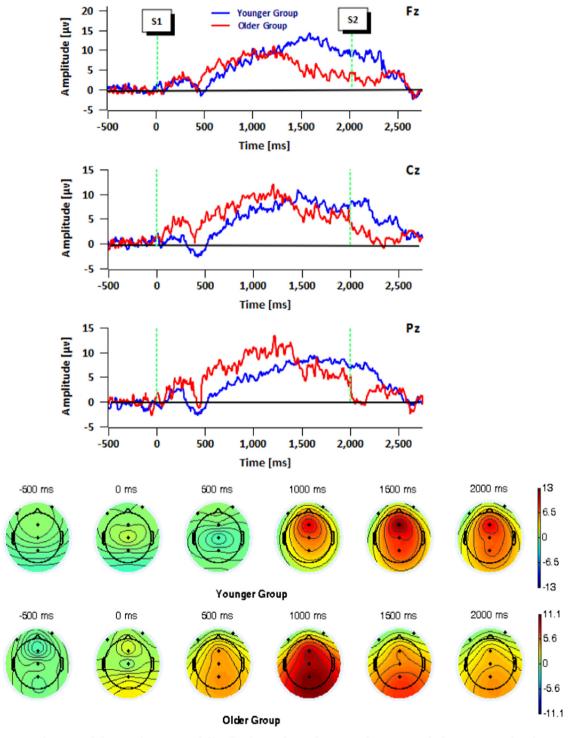


Fig. 2 – The waveforms and the spatio-temporal distributions of grand averaged CNV recorded at Fz, Cz and Pz in younger and older group. Baseline is –500–0 ms. The vertical green line at 0 ms represents the warning stimulus (S1) and at 2000 ms represents response stimulus (S2). Each trace shows the mean value of the amplitudes averaged across all subjects in both groups. In scalp topographies, the electrodes have marked in filled circles on the scalp.

Columbus, OH, USA) at a sampling frequency of 500 Hz. The X and Y axis of force platform were aligned in the direction of mediolateral and anteroposterior progression of subjects, respectively.

5.6. Data analysis

5.6.1. EEG analysis

EEG signal epochs were extracted, and then time-locked to the onset of S1 from -500 to 2500 ms, with a 500 ms pre-S1 window served as a baseline. Signal epochs contaminated with artifacts (such as muscle activities or eye movements) were excluded from further analysis using EEGLAB 7.1.4 (sccn. ucsd.edu/eeglab), a signal processing environment that decomposes artifact contaminated EEG from electrocortical activity using Independent Component Analysis. Generally, younger group contained 288 artifact -free epochs and older group contained 259 epochs, reflecting 10% and 14% of epochs

in younger and older group were excluded from analysis, respectively. Moreover, subjects who had less than 50% of epochs accepted were excluded from further analysis. The artifact -free signals were baseline corrected and ensembleaveraged, for each electrode and each subject, to enhance the signal-to-noise ratio. Subsequently, for each ensembleaveraged signal, the mean amplitude 200-ms prior to S2 were computed and considered as late CNV amplitude. Further, since the EEG signals exceeded the baseline about 500 and 400 ms after S1 in the younger and older subjects, respectively. Accordingly, the maximal potential from 500 ms after S1 to S2 was specified as the CNV peak, and its amplitude from baseline (CNV peak amplitude) and its latency relative to S2 (CNV peak time) were measured (Fujiwara et al., 2012). Fig. 2 presents the waveforms and the spatio-temporal distributions of grand averaged CNV recorded at Fz, Cz and Pz for groups.

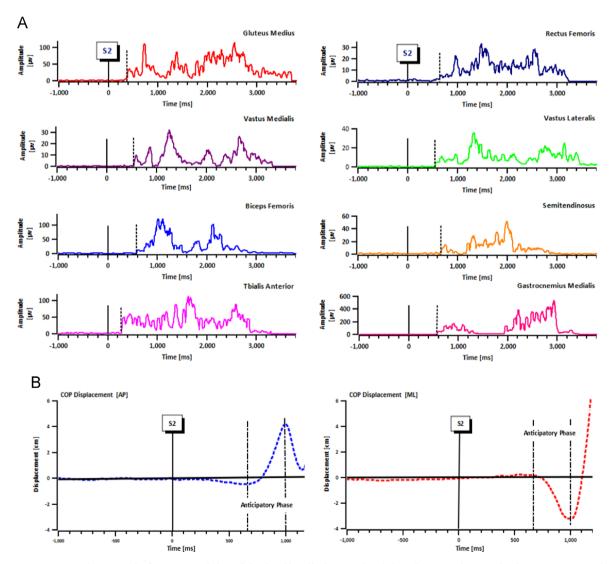


Fig. 3 – A representative sample for EMG activity of the leading limb muscles (A) and COP trajectory in the anteroposterior (AP) and mediolateral (ML) directions in a young subject (B). Time 0 ms denotes the start of response stimulus (S2). In (A), dotted line represents the onset of muscle activities and in (B) shows the onset of COP displacement and the most postrolateral position of the COP trajectory beneath the leading limb, respectively.

5.6.2. EMG analysis

In the EMG analysis, the purpose was to measure the onset of muscle activity. Thus, at first, the raw data were high-pass filtered with a zero-phase shift, 6th-order Butterworth, with a cut off frequency of 30 Hz to remove movement artifacts. Then, root mean square (RMS) values were taken with a moving window 50 ms. The onset of muscles was determined as the instant at which the EMG deviated more than the mean +2 SDs of the background activity during 200 ms before S2 for a period >50 ms. Onset time of muscles was measured from this point to S2 and was used for analysis (Maekawa et al., 2013). All analysis was performed by custom-written scripts in MATLAB version 8 (MathWorks Inc., Natick, MA, USA). Fig. 3A presents a sample of recorded EMG from leading limb muscles in a representative younger subject.

5.6.3. Force plate analysis

The raw data were low-pass filtered by a zero-phase shift, 6th-order Butterworth, with a cut-off frequency of 10 Hz. Onset of COP displacement was defined as the instant at which the vertical GRF deviated more than the mean +2 SDs of the force during the first 500 ms of quiet stance (Hass et al., 2012) and time to onset COP displacement was measured from appearance of response stimulus (S2) to this instant. Also, the time interval between the onset point and the most postrolateral position of the COP trajectory beneath the leading limb was defined as the anticipatory phase. In this phase, the mean COP displacements and the velocities were computed in the anteroposterior (AP) and mediolateral (ML) directions. All data was processed in MATLAB version 8 (MathWorks Inc., Natick, MA, USA). Fig. 3B shows a sample of recorded COP trajectory in the AP and ML directions.

5.6.4. Statistical analysis

The Kolmogorov–Smirnov test determined that all data would satisfy the assumptions of normality. Therefore, independent t-tests were performed to reveal the presence of significant differences between groups. Also, the level of association between the parameters for both groups was explored using Pearson's product moment correlation coefficients. Correlation coefficients greater than 0.5 according to Cohen's criteria were considered as good to excellent associations (small: r=0.10–0.29, medium: r=0.30–0.49, and large: r=0.50–1.0). All statistical calculations were conducted using SPSS version 16.0 (SPSS Inc, Chicago IL, USA) and statistical significance threshold was set at 0.05 for all tests.

Conflict of interest

There is no conflict of interest related to this study.

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