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CONTRIBUTION OF THE SUPPLEMENTARY MOTOR AREA AND THE CEREBELLUM TO THE ANTICIPATORY POSTURAL ADJUSTMENTS AND EXECUTION PHASES OF HUMAN GAIT INITIATION

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Abstract—Several brain structures including the brainstem, the cerebellum and the frontal cortico-basal ganglia network, with the primary and premotor areas have been shown to participate in the functional organization of gait initiation and postural control in humans, but their respective roles remain poorly understood. The aim of this study was to better understand the role of the supplementary motor area (SMA) and posterior cerebellum in the gait initiation process. Gait initiation parameters were recorded in 22 controls both before and after continuous theta burst transcranial stimulation (cTBS) of the SMA and cerebellum, and were compared to sham stimulation, using a randomized double-blind design study. The two phases of gait initiation process were analyzed: anticipatory postural adjustments (APAs) and execution, with recordings of soleus and tibialis anterior muscles. Functional inhibition of the SMA led to a shortened APA phase duration with advanced and increased muscle activity; during execution, it also advanced muscle co-activation and decreased the duration of stance soleus activity. Cerebellar functional inhibition did not influence the APA phase duration and amplitude but increased muscle co-activation, it decreased execution duration and showed a trend to increase velocity, with increased swing soleus muscle duration and activity. The results suggest that the SMA contributes to both the timing and amplitude of the APAs with no influence on step execution and the posterior cerebellum in the coupling between the APAs and execution phases and leg muscle activity pattern during gait initiation.

Key words: gait initiation, anticipatory postural adjustments, supplementary motor area, cerebellum, transcranial magnetic stimulation.

INTRODUCTION

In humans, gait initiation is particularly challenging for motor and postural control as the subject has to simultaneously perform a whole-body forward movement and pass from a stable (double-leg stance) to an unstable position (single-leg stance). It is associated with anticipatory postural adjustments (APAs) with a backward and lateral shift of the center of foot pressure (CoP) toward the swing leg, in relation with activation of both tibialis anterior (TA) muscles, allowing the swing leg to lift. The neural substrates for APAs' generation and step execution are not fully known. In animals, locomotion can be initiated by electrical or chemical activation of the supplementary motor area at the cortical level, and the subthalamic, the mesencephalic locomotor and cerebellar midline regions at the subcortical level (Mori et al., 1989; Takakusaki et al., 2004). In humans, few studies have examined the role of these brain regions in the gait initiation process. However, repetitive transcranial magnetic stimulation applied above the SMA provokes a shortening of the APA duration of the first step after stimulation with no change in APA amplitude (Jacobs et al., 2009a). Startle stimuli, thought to modulate pontomesencephalic reticular formation activity, shorten APAs with no change in the step execution characteristics (Queralt et al., 2010). Acoustic stimuli can also elicit APAs without being followed by step execution (Delval et al., 2012), but only when the subject is expecting to initiate a step, suggesting that the brainstem releases APAs depending on the cognitive context (to walk or not) (MacKinnon et al., 2007).

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Abbreviations: APAs, anticipatory postural adjustment; CoG, center of gravity; CoP, center of foot pressure; EC, eyes closed; EO, eyes opened; FC, foot contact; FO, foot off; rTMS, repetitive transcranial magnetic stimulation; SMA, supplementary motor area; SOL, soleus muscle; TA, tibialis anterior muscle; TBS, theta burst stimulation.

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Lastly, transcranial direct current stimulation applied over the cerebellum affects spatial characteristics of walking during locomotor adaptation (Jayaram et al., 2012), but the effects of cerebellar stimulation on APAs generation and gait initiation have not been studied. In patients with lesions and/or dysfunction of these different brain regions, various gait initiation deficits have also been reported. In patients with premotor cortical areas involving the SMA, a lack of gait initiation (or freezing of gait-FOG phenomenon) has been reported with an inability to lift the feet from the ground and execute the first step (Nutt et al., 1993; Nadeau, 2007; Bartels and Leenders, 2008). Such a FOG phenomenon is also observed in patients with mesencephalic locomotor region (MLR) lesions (Masdeu et al., 1994) and people with Parkinson's disease (PD) (Giladi et al., 1997) and has been linked to altered APAs prior to gait initiation (Jacobs et al., 2009b) with reduced SMA-MLR connectivity (Halliday et al., 1998; Okada et al., 2011; Gallea et al., 2017). Patients with cerebellar lesions or dysfunction show increased stride width and shorter stride length with longer duration and increased lower leg muscle activity and co-activation (Timmann et al., 2000); with abnormal temporal organization of the muscular pattern (Martino et al., 2014; Bruttini et al., 2015). Recently, distorted APAs prior to step execution and altered postural control during walking with shorter and irregular steps (Morton and Bastian, 2007; Fernandez et al., 2013) have been reported in patients suffering from essential tremor (ET), these patients presented with posterior cerebellar atrophy (Gallea et al., 2015). Taken together these data support the involvement of the cortico (SMA)-pontine-cerebello-thalamo-cortical pathway in APAs generation and the gait initiation process.

In order to provide a more detailed understanding of how the cortico (SMA)-pontine-cerebello-thalamo-cortical pathway contributes to the preparation and the execution of the first step in humans, we selectively disrupted the SMA and the cerebellum with continuous theta burst repetitive transcranial magnetic stimulation (cTBS) and evaluated the effects of the stimulation on the APAs and execution phases of gait initiation. Consistent with previous studies, we hypothesized that the SMA would be mainly involved in generating the APAs to lift the leg from the ground while the cerebellum would adjust the postural balance and foot placement during walking.

EXPERIMENTAL PROCEDURES

Participants

Twenty-two healthy volunteers (14 M/8 F, mean age ± SD = 29.5 ± 7.3 years) were included in this study which received approval from the local ethics committee (Paris VI University) and was promoted by the INSERM (C12-05, N° ID RCB 2012-A00796-37, ClinicalTrials.gov Registration NCT02976298). All the participants gave written informed consent to participate. Exclusion criteria were any history of peripheral neurological or orthopedic disease, and of central nervous systems or psychotropic drug use or contraindication to rTMS (Rossi et al., 2009).

Gait initiation protocol

Subjects, barefoot, and standing upright and motionless on a force plate (0.9 × 1.8 m, Advanced Mechanical Technology Inc. LG6-4-1, USA) were instructed to commence walking for 5 m (5–7 steps per trial) following an auditory cue. The outlines of the participants' feet were recorded to ensure that stance width remained consistent throughout the experiments. In each trial, the first two steps were captured for analysis. The subjects were instructed to walk at their usual self-paced speed. Each subject walked a total of 10 trials before and after rTMS. The subjects performed separate sessions in a randomized order for rTMS over the SMA, cerebellum and sham stimulation (to either SMA or cerebellum). The sessions were separated by at least 7 days.

RTMS PROTOCOL

The intensity of stimulation applied to SMA or cerebellum was chosen relative to the active motor thresholds (aMT) of the right tibialis anterior (TA) or right abductor pollicis brevis (APB) muscles respectively, as previously reported (Gerloff et al., 1997; Popa et al., 2013). The rationale for using TA muscle representation as hotspots for adjusting SMA stimulation intensity was that leg representations in the SMA and the primary motor cortex are located in adjacent positions and at a similar depth in the interhemispheric fissure (Gerloff et al., 1997). Accordingly stimulation sessions started by identifying the motor 'hot spot' of either the right TA or right APB muscles according to the session and calculating their aMT using standard procedures (Rossini et al., 1994; Rothwell, 1997). To that end TMS pulses were applied using a 70-mm figure-of-eight coil connected to a Rapid2 magnetic stimulator (Magstim Company). Induced currents were biphasic and directed posterior to anterior (Arai et al., 2012). For the TA muscle, the coil was held on or close to the midline with the handle pointing backward. For the APB muscle, the coil was held at ~45° from the midline for optimal trans-synaptic activation of the motor cortex (Werhahn et al., 1994; Kaneko et al., 1996).

The SMA and cerebellum targets were marked on the individual brain MRIs by the use of an MRI-based neuronavigation system (eXimia 2.2.0, Nextstim Ltd). For the SMA, the target was the SMA proper and marked on the MRI relative to a vertical line from the anterior commissure perpendicular to the antero-posterior commissure line in the sagittal place, which is a standardized separator for the SMA proper and the pre-SMA (Picard and Strick, 1996; Zilles et al., 1996; Vorobiev et al., 1998), The center of the coil was positioned over the target, with the coil handle pointing backward so that the induced current had a posterior–anterior orientation. For the cerebellum, stimulation was applied consecutively to each side targeting lobule VIII of the cerebellum (Popa et al., 2010, 2013), with the coil handle pointing upward so that the induced current had a caudal to rostral orientation.

Continuous theta-burst stimulation (cTBS) protocol (Huang et al., 2005) was used with 600 stimuli delivered at 80% TA aMT (for SMA) or 80% APB aMT

(for cerebellum) in three-pulse bursts at 50 Hz, repeated every 200 ms continuously for 40 s (5 Hz). For sham stimulation (delivered with a cTBS pattern), we used a 'sham' coil that delivered only 10% of the intensity. The stimulation intensities used in this study are well below the maximum limit recommended by current rTMS safety guidelines (Rossi et al., 2009).

Data collection and analysis

The investigators analyzing kinematic, dynamic and EMG data were blinded to the stimulation conditions.

Kinetic and dynamic data. The spatial and temporal kinetics parameters, i.e. acceleration and velocity of the center of gravity (CoG) and displacements of center of foot pressure (CoP), were extracted from the force platform data in real time and displayed in a custom-built MATLAB interface (Chastan et al., 2010). The force platform analog signals were sampled at 1000 Hz.

The onset of the APAs was defined as the moment of the first biomechanical event (t0) with posterior and lateral CoP displacement (Fig. 1), and its end as the moment of the foot-off of the swing leg (FO1). The APAs phase was divided in 2 distinct phases (Hass et al., 2004): the S1 phase starting at t0 and ending at the time of the maximum posterior and lateral CoP shift, and the S2 phase starting at the onset of the lateral shift of the CoP in the opposite direction toward the stance limb and ending when the CoP is in its most lateral and posterior position under the stance limb (Fig. 1). The APAs duration and maximum posterior and lateral CoP displacements were measured.

The step execution phase was defined as the period between FO1 and the foot-off of the stance leg (FO2). The step length, step width, execution velocity and duration of the swing (time between FO1 and the foot-contact of the stance leg – FC1) and double-stance (period between the foot contact of the swing leg – FC1 and FO2) phases were calculated (Fig. 1). Two values were extracted from the vertical CoG velocity: the peak negative value during the swing phase (V1) and its value at the time of foot contact (V2), allowing us to calculate the braking index ((V1-V2)/V1*100), which reflects active postural control (Welter et al., 2007).

EMG data. Electromyographic (EMG) activity of the Soleus (SOL) and Tibialis anterior (TA) muscles were recorded with surface bipolar electrodes according to SENIAM procedure (Hermens et al., 2000) (WAVE Plus, Ref. WP180, Cometa srl, Italy). The EMG signals were band-pass filtered at 30–300 Hz (6th order Butterworth filter), and rectification and low-pass filtering at 50 Hz (2nd order Butterworth, zero-phase forward and reverse filter) were applied. Muscular bursts were detected by the Teager–Kaiser energy operator method by calculating the energy of the signal (Solnik et al., 2010). The linear envelopes of each muscular burst were measured, normalized to the maximum peak amplitude in each gait condition and the root mean square (RMS) calculated (Barzilay and Wolf, 2011). The onset, duration of each SOL and TA muscles burst and co-contraction (simultaneous SOL

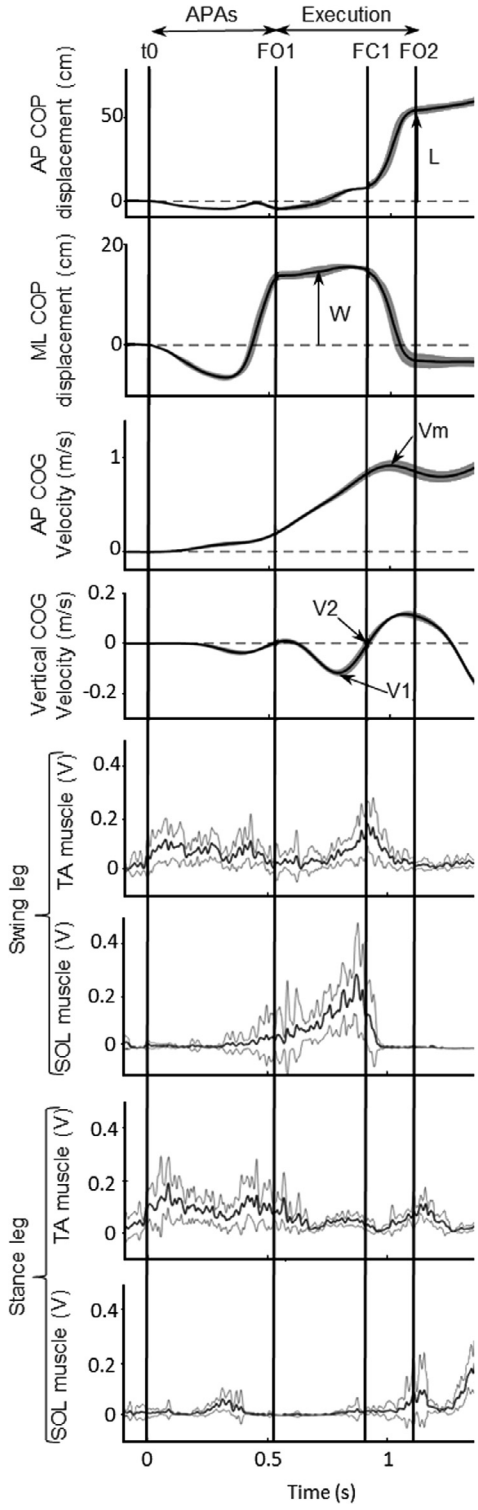

Fig. 1. Biomechanical parameters and leg muscle activity during gait initiation in a control subject. Curves represent the smoothed mean of ten trials and standard deviation. The rectified electromyographic activity of the tibialis anterior (TA) and soleus (SOL) muscles of the swing and the stance legs is shown in the four lower panels. AP: antero-posterior; CoG: center of gravity; CoP center of pressure; t0: time of the first biomechanical event; FO1: foot-off of the swing leg; FC1: foot contact of the swing leg; ML: medio-lateral CoP displacement; L: length of the first step; Vm: peak antero-posterior velocity of the center of gravity; V1: peak negative value of CoG vertical velocity; V2: value of CoG vertical velocity at the time of FC1.

and TA muscles activity) were calculated. We calculated a ratio of co-contraction by dividing the linear envelope of SOL or TA muscle activity with the sum of the SOL and TA linear envelopes (Hesse et al., 2000).

Statistical analysis

For each stimulation condition, we employed a linear mixed effects model with two fixed effects: stimulation (before stimulation, sham-cTBS and cerebellar-cTBS or SMA-cTBS) and day (chronological order of sessions), adding a random effect on subjects to take into account the inter-subject variability. When a significant main effect was seen, the Tukey–Kramer test was used for multiple comparisons. Statistical analyses were performed using R (version 3.2.2). The significance level was taken as $p < 0.05$ with Bonferroni's correction. We considered as significant the changes induced by the cTBS only if there was a significant difference between the values obtained before *versus* after the stimulation and a difference between the values obtained after active *versus* sham cTBS. We considered as a trend the changes induced by cTBS if there was a significant difference between the values obtained before *versus* after the stimulation or after active *versus* sham cTBS, but no difference between the values obtained at baseline and after sham stimulation.

RESULTS

Effects of functional inactivation of the SMA or cerebellum on the APAs phase

With SMA-cTBS, the APAs' phase duration significantly decreased, the swing SOL muscle activity started significantly earlier and the stance TA muscle RMS activity significantly increased (Fig. 2). In comparison to baseline, after SMA-cTBS, the S2 phase duration tended to decrease and the stance SOL muscle activity to start earlier. Lastly, with SMA-cTBS in comparison to sham stimulation, the lateral CoP displacement tended to increase and the swing SOL muscle activity tended to be activated longer and with increased RMS amplitude (Table 1).

With cerebellar-cTBS, the amplitude and timing of APAs were not significantly modified, but the duration of the TA and SOL muscle co-activation of the stance leg significantly increased (Table 2). After cerebellar-cTBS, the stance TA muscle activity tended to start later in comparison to baseline, and the duration of the swing and stance SOL muscle activity tended to increase. Lastly, with cerebellar-cTBS the lateral CoP shift tended to decrease in comparison to sham stimulation, (Table 2).

Effect of functional inhibition of the SMA or cerebellum on step execution

With SMA-cTBS, the execution and double-stance durations, step length, execution velocity (Fig. 2) and braking index (not shown) were not significantly modified. The stance SOL muscle activity duration significantly decreased (Fig. 2). In comparison to baseline, the swing TA muscle activity duration tended to decrease (Fig. 2) and the swing SOL-TA muscle co-activation to start earlier (Table 3).

With cerebellar-cTBS, the execution duration was significantly lower with no significant change in the double-stance duration, step length, execution velocity (Fig. 2) or braking index (not shown). The swing SOL muscle activity duration was significantly increased (Fig. 2). With cerebellar-cTBS, the swing SOL muscle activity RMS amplitude tended to increase in comparison to baseline, with reduced swing TA muscle RMS amplitude (Table 4). Lastly, with cerebellar-cTBS the swing TA muscle activity duration tended to decrease in comparison to sham stimulation (Fig. 2).

DISCUSSION

In the present study, we investigated how the APAs and step execution could be modified by cTBS over the SMA and the cerebellum in healthy subjects. Shorter APAs were produced by cTBS over the SMA with earlier activation of swing SOL muscle, with no significant changes in the step execution parameters, except for a shorter stance SOL muscle activation. Conversely, no significant change in the APAs timings and amplitudes were induced by cTBS over the cerebellum but an increased stance SOL-TA muscle co-activation duration during APAs and a shorter step execution duration with longer swing SOL muscle activation. Neither SMA nor cerebellar-cTBS provoked significant changes in the step length and velocity or postural control during gait execution (i.e. double-stance duration and braking index).

In this study, we used the classical parameters used for cTBS, i.e. 0.8 aMT, 600 pulses, thought to induce a transient decrease in synaptic efficacy in the targeted area lasting approximately 30 min after stimulation (Huang et al., 2005), through a combination of long-term depression and long-term potentiation mechanisms (Huang et al., 2005; Stagg et al., 2009), with, however, a significant inter-subject variability. In our study, cTBS of the SMA led probably to a reduction in its output signal that provoked increased APAs performance with changes both in the timing (shortening) and amplitude (tendency to increase CoP displacements) of the APAs, related to an earlier and greater activation of TA muscles (Mann et al., 1979). Our results are in line with reports of reduced APAs durations prior to step initiation with 1-Hz rTMS of the SMA (Jacobs et al., 2009a). This effect could result from disruption of sensory perception of self-generated actions (Haggard and Whitford, 2004), in addition to changes in perception threshold (Legon et al., 2013), leading to decreased effort perception (Zenon et al., 2015) with, in consequence, an increased force production and increased muscle activity (our results). Another explanation would be that SMA disruption interferes with the automatic inhibitory control of motor responses (Albares et al., 2014). The finding that SMA disruption significantly improves akinesia (Eggers et al., 2015) and decreases abnormally prolonged APAs phase durations prior to gait initiation (Jacobs et al., 2009a) in PD patients,

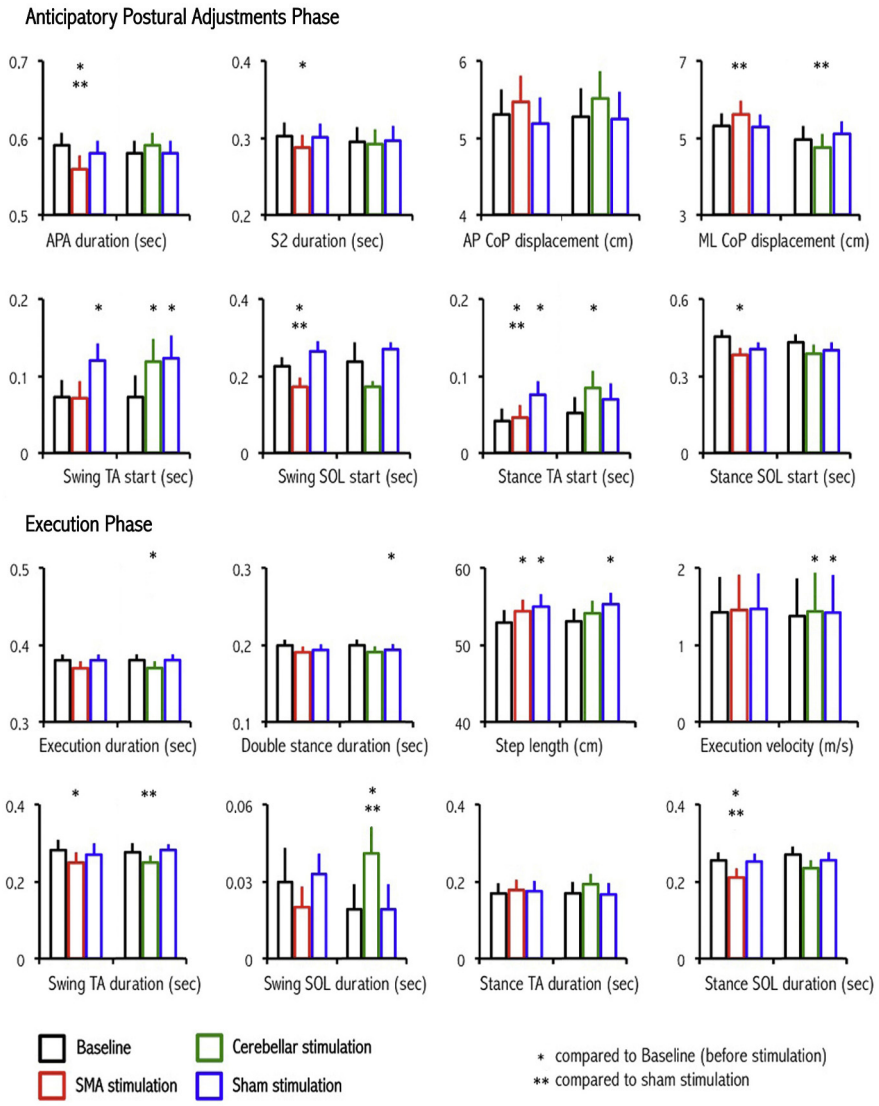

Fig. 2. Effects of SMA and cerebellar stimulation on gait initiation in healthy subjects. Biomechanical parameters and electromyographic activity during gait initiation before (baseline; black), after SMA (red) or cerebellar (green) theta burst stimulation and after sham stimulation (blue). Upper panel: anticipatory postural adjustments phase; lower panel: execution phase.

is also in line with our results. In contrast, anodal transcranial direct current stimulation (tDCS) of the SMA (thought to induce an excitation) provokes an increase in the APAs developing in the biceps and the triceps brachii during brisk index-finger flexions, without changes in prime mover muscle recruitment and index-finger kinematics (Bolzoni et al., 2015). Lastly, we observed no significant change in balance control during gait after functional inhibition of the SMA suggesting that the SMA is not specifically involved in dynamic postural control while walking, *per se*, but more in internal representation of sensory information regarding postural status (Jacobs and Horak, 2007).

cTBS of the cerebellum produced no marked effects on the spatiotemporal APAs parameters but altered the leg muscle pattern and increased intra-limb muscle co-activation. Our present findings are in line with the fact that anodal tDCS applied to the lateral cerebellum does not modify locomotor patterns, except in the case of adaptive learning (Jayaram et al., 2011, 2012), whereas posterior cerebellar disruption provokes maladjustment of sensorimotor calibration with a decoupling between the motor component of the movement (execution) and the afferent sensory information (postural adjustment) (Hubsch et al., 2013) with an alteration of muscle activation pattern with increased co-activation of agonist and antagonist muscles during movement (Mari et al., 2014). The changes in muscle activation pattern during the APAs or the first-step execution induced by cerebellar TBS were probably mediated through the cerebello-thalamo-projections to the motor areas as we targeted lobule VIII of the cerebellum (Popa et al., 2010), this lobule being activated during sensory–motor tasks (Stoodley et al., 2012) in connection with the premotor and motor cortical regions (O'Reilly et al., 2010). The fact that, in our experiment, cTBS of the cerebellum has little or no effect on postural control suggests that we did not significantly modified vermis or vestibular cerebellum activity (Inukai et al., 2016).

We took several precautions to ensure reliable findings. Subjects were randomly assigned to begin with sham, or cerebellar or SMA stimulation to minimize any possible order effects. By having the sessions scheduled at least one week apart we excluded a 'take over' effect. We used a within-subject design and a sham condition to limit the influence of the known large inter-subject variability in susceptibility to develop TBS-induced plasticity (Lopez-Alonso et al., 2014; Vallence et al., 2015) and stimulation of SMA and the lobule VIII of the cerebellum were both performed under neuro-navigation control. Despite this precaution, we observed changes in gait initiation parameters after sham stimulation. This could have led to underpowered results. An unsolved issue is how to assess the efficacy of the cerebellum or SMA theta-burst stimulation in individuals and consequently, how to determine the sample size needed for such experiments. Stimulation intensity was adjusted according to the individual's motor cortex excitability yet it is unclear to what extent M1 excitability reflects cerebellar or SMA excitability. Lastly, we could not exclude the possibility that stimulation-induced effects on EMG pattern and amplitude occurred by influencing the tonic

Table 1. Effects of SMA theta-burst stimulation on Soleus and Tibialis Anterior muscle activity during the APAs prior to gait initiation in 22 healthy subjects

		SMA cTBS		
		Before TBS	SMA-TBS	Sham-TBS
Swing Leg				
TA muscle	Duration (ms)	400 ± 42	381 ± 43	382 ± 43
	RMS amplitude	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.01
SOL muscle	Duration (ms)	100 ± 21	124 ± 21**	67 ± 21*
	RMS amplitude	0.05 ± 0.01	0.06 ± 0.01**	0.03 ± 0.01*
Co-contraction	Start (ms)	157 ± 32	129 ± 33**	240 ± 33*
	Duration (ms)	83 ± 21	83 ± 22	53 ± 22*
	Ratio (%)	14 ± 3	16 ± 3	15 ± 3
Stance Leg				
TA muscle	Duration (ms)	372 ± 38	373 ± 39**	315 ± 38*
	RMS amplitude	0.17 ± 0.02	0.19 ± 0.02 * **	0.15 ± 0.02
SOL muscle	Duration (ms)	83 ± 21	97 ± 21	111 ± 21
	RMS amplitude	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Co-contraction	Start (ms)	349 ± 53	345 ± 55	370 ± 56
	Duration (ms)	48 ± 14	46 ± 14	30 ± 14
	Ratio (%)	9 ± 2	10 ± 2	9 ± 2

Results are expressed as mean ± SD. RMS: root mean square; SMA: supplementary motor area; SOL: soleus muscle; TA: tibialis anterior muscle; TBS: theta burst stimulation.

* $P < 0.05$ when compared to before SMA-TBS.

** $P < 0.05$ when compared to sham-TBS.

Table 2. Effects of cerebellar theta-burst stimulation on Soleus and Tibialis Anterior muscle activity during the APAs prior to gait initiation in 22 healthy subjects

		Cerebellar cTBS		
		Before TBS	CER-TBS	Sham-TBS
Swing Leg				
TA muscle	Duration (ms)	400 ± 40	381 ± 41	386 ± 41
	RMS amplitude	014 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
SOL muscle	Duration (ms)	94 ± 21	115 ± 22**	62 ± 22*
	RMS amplitude	0.05 ± 0.01	0.05 ± 0.01**	0.03 ± 0.01*
Co-contraction	Start (ms)	176 ± 34	228 ± 36	240 ± 35*
	Duration (ms)	85 ± 21	62 ± 21	57 ± 21*
	Ratio (%)	15 ± 3	14 ± 3	15 ± 3
Stance Leg				
TA muscle	Duration (ms)	359 ± 39	346 ± 39	326 ± 39
	RMS amplitude	0.17 ± 0.02	0.16 ± 0.02	0.16 ± 0.02
SOL muscle	Duration (ms)	80 ± 17	117 ± 18*	113 ± 18
	RMS amplitude	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Co-contraction	Start (ms)	320 ± 62	355 ± 63	352 ± 65
	Duration (ms)	51 ± 19	85 ± 20 * **	36 ± 20
	Ratio (%)	9 ± 3	12 ± 3	9 ± 3

Results are expressed as mean ± SD. CER: cerebellum; RMS: root mean square; SOL: soleus muscle; TA: tibialis anterior muscle; TBS: theta burst stimulation.

* $P < 0.05$ when compared to before SAM-TBS.

** $P < 0.05$ when compared to sham-TBS.

descending drive exerted on the spinal motoneurons through the descending cerebellar vestibular and reticulospinal tracts. The fact that cerebellar stimulation with single-TMS pulses facilitates the ipsilateral soleus H reflex without increasing the soleus electromyographic activity goes against this hypothesis, however (Matsugi et al., 2014).

CONCLUSION

In summary, the results support distinct roles for the SMA and the lateral posterior cerebellum in human gait initiation, with the SMA coding for the timing, and probably amplitude, of the preparatory phase of the gait initiation, and the posterior cerebellum contributing to the inter- and intra-limb muscle coordination, and

Table 3. Effects of SMA theta-burst stimulation on Soleus and Tibialis Anterior muscle activity during first step execution in 22 healthy subjects

		SMA cTBS		
		Before TBS	SMA-TBS	Sham-TBS
Swing Leg				
TA muscle	Start (ms)	50 ± 14	57 ± 15	55 ± 15
	RMS amplitude	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
SOL muscle	Start (ms)	177 ± 39	180 ± 41	157 ± 41
	RMS amplitude	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Co-contraction	Start (ms)	203 ± 47	98 ± 52*	165 ± 48
	Duration (ms)	16 ± 8	9 ± 8**	35 ± 8*
	Ratio (%)	6 ± 3	3 ± 3**	9 ± 3*
Stance Leg				
TA muscle	Start (ms)	92 ± 19	75 ± 20	105 ± 20
	RMS amplitude	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01
SOL muscle	Start (ms)	61 ± 14	75 ± 14	65 ± 14
	RMS amplitude	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.01*
Co-contraction	Start (ms)	123 ± 20	138 ± 21	135 ± 21
	Duration (ms)	103 ± 23	100 ± 24	100 ± 24
	Ratio (%)	12 ± 3	12 ± 3	15 ± 3

Results are expressed as mean ± SD. RMS: root mean square; SMA: supplementary motor area; SOL: soleus muscle; TA: tibialis anterior muscle; TBS: theta burst stimulation.

* $P < 0.05$ when compared to before SMA-TBS.

** $P < 0.05$ when compared to before sham-TBS.

Table 4. Effects of cerebellar theta-burst stimulation on Soleus and Tibialis Anterior muscle activity during first step execution in 22 healthy subjects

		Cerebellar cTBS		
		Before TBS	CER-TBS	Sham-TBS
Swing Leg				
TA muscle	Start (ms)	55 ± 11	46 ± 12	50 ± 12
	RMS amplitude	0.12 ± 0.01	0.11 ± 0.01**	0.14 ± 0.01*
SOL muscle	Start (ms)	208 ± 36	203 ± 37	223 ± 39
	RMS amplitude	0.02 ± 0.01	0.03 ± 0.01*	0.02 ± 0.01
Co-contraction	Start (ms)	139 ± 31	139 ± 31	137 ± 31
	Duration (ms)	20 ± 8	26 ± 9	33 ± 9
	Ratio (%)	7 ± 2	8 ± 2	9 ± 2*
Stance Leg				
TA muscle	Start (ms)	73 ± 20	87 ± 21	104 ± 21
	RMS amplitude	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
SOL muscle	Start (ms)	56 ± 13	50 ± 13	59 ± 13
	RMS amplitude	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.01*
Co-contraction	Start (ms)	106 ± 20	110 ± 21	123 ± 21
	Duration (ms)	109 ± 24	116 ± 25	98 ± 25
	Ratio (%)	15 ± 3	16 ± 3	14 ± 3

Results are expressed as mean ± SD. CER: cerebellum; RMS: root mean square; SOL: soleus muscle; TA: tibialis anterior muscle; TBS: theta burst stimulation.

* $P < 0.05$ when compared to before cerebellar-TBS.

** $P < 0.05$ when compared to before sham-TBS.

probably coupling between the APAs and the execution phases. Further studies are needed to examine the potential therapeutic effect of such non-invasive cerebral stimulation in patients suffering from SMA or cerebellar dysfunction.

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AUTHORS' CONTRIBUTIONS

A.R, X.D, A.V.H and M.-L.W. performed experiments; A. R, X.D, A.V.H, J.-L.G and M.-L.W analyzed data; A.R,

A.V.H, S.M. and M.-L.W interpreted results of experiments; A.R; A.V.H. and M.L.W prepared figures; A.R., A.V.H., S.M and M.-L.W. drafted, edited and revised manuscript; S.M. and M.L.W. conception and design of research.

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