CHAPTER

4

Assessment of transmission in specific descending pathways in relation to gait and balance following spinal cord injury

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Abstract

Human bipedal gait requires supraspinal control and gait is consequently severely impaired in most persons with spinal cord injury (SCI). Little is known of the contribution of lesion of specific descending pathways to the clinical manifestations of gait deficits. Here, we assessed transmission in descending pathways using imaging and electrophysiological techniques and correlated them with clinical measures of impaired gait in persons with SCI.

Twenty-five persons with SCI participated in the study. Functional assessment of gait included the Walking Index for Spinal Cord Injury (WISCI), the Timed-Up and Go (TUG), the 6-Min Walking Test (6MWT), and the maximal treadmill gait speed. Balance was evaluated clinically by the Berg Balance Scale (BBS). The amplitude of tibialis anterior (TA) motor-evoked potentials (MEPs) at rest elicited by transcranial magnetic stimulation as a measure of corticospinal transmission showed a moderately good correlation with all clinical measures ($r^2 \sim 0.5$), whereas the latency of the MEPs showed less good correlation ($r^2 \sim 0.35$). Interestingly, the MEP amplitude was correlated to atrophy in the ventrolateral rather than the dorsolateral section of the spinal

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cord where the main part of the corticospinal tract is located. TA intramuscular coherence in the beta and gamma frequency range has been suggested to reflect corticospinal transmission and was, consistent with this, found to be correlated to atrophy in the dorsolateral and ventrolateral sections of the spinal cord. Coherence was found to correlate to all clinical measures to the same extent as the MEP amplitude. The latency and duration of medium-latency responses in the soleus muscle to galvanic stimulation as measures of vestibulospinal transmission showed very good correlation to BBS ($r^2 = -0.8$) and moderately good correlation to the assessments of gait function ($r^2 \sim 0.4$). 6MWT and gait speed were correlated to atrophy of the lateral sections of the spinal cord bilaterally, whereas BBS was correlated to atrophy of both lateral and ventral sections of the spinal cord. No significant correlation was observed between the electrophysiological tests of corticospinal and vestibulospinal transmission.

Combination of different electrophysiological and anatomical measures using best subset regression analysis revealed improved prediction of gait ability, especially in the case of WISCI.

These findings illustrate that lesion of corticospinal and vestibulospinal pathways makes different contributions to impaired gait ability and balance following SCI and that no single electrophysiological or anatomical measure provide an optimal prediction of clinical gait and balance disability. We suggest using a combination of anatomical and electrophysiological measures when evaluating spinal cord integrity following SCI.

Keywords

corticospinal tract, locomotion, transcranial magnetic stimulation, incomplete spinal cord injured patients, vestibulospinal tract, galvanic vestibular stimulation, clinical test, balance, MRI, atrophy

1 INTRODUCTION

Gait is a complex motor task, which requires integration of central motor commands and sensory feedback signals at multiple levels of the neuroaxis (Nielsen, 2003; Yang and Gorassini, 2006). It is difficult to establish direct conclusive evidence that spinal networks are involved in generating the basic locomotor rhythmicity in humans, as is the case during quadriplegic locomotion in animals (Fedirchuk et al., 1998; Grillner and Wallen, 1985). However, there is ample evidence that such rhythm-generating networks exist and may be activated under certain circumstances (Calancie et al., 1994; Dietz et al., 1995; Gerasimenko et al., 2008; Minassian et al., 2004). There is also direct evidence demonstrating that sensory feedback mechanisms contribute through spinal networks in the generation of muscle activity during human gait (af Klint et al., 2008, 2010a,b; Grey et al., 2007; Sinkjaer et al., 2000). Proper functioning of these networks during human gait appears to rely to a larger extent on intact supraspinal control than what is the case in animals (Eidelberg et al., 1981; Fedirchuk et al., 1998). In humans, complete lesion of descending motor pathways is associated with paralysis, and functional gait ability, in contrast to quadriplegic animals, never recovers in these cases (Dietz, 1995; Dietz et al., 1995).

Severe gait disability is also common following incomplete lesions, and recovery of function appears to depend largely on the recovery of supraspinal control of the spinal circuitry (Hubli and Dietz, 2013; Knikou, 2012). In able-bodied humans, corticospinal control of the spinal circuitries has also been shown to be involved in the control of uncomplicated treadmill walking and contribute directly to the generation of muscle activity (Barthelemy and Nielsen, 2010; Barthelemy et al., 2011; Petersen et al., 2001, 2012).

The deficits of gait following spinal cord injury (SCI) are varied and involve muscle weakness that affect either the whole limb or movement at specific joints, coordination of muscle activity within each leg or between the two legs, and reduced balance (Dietz, 2013). Knowledge of the relation between these clinical gait deficits and the underlying lesion of specific descending supraspinal pathways is of both basic and clinical scientific interest. We have limited knowledge of how transmission in specific descending pathways is integrated with activity in spinal neuronal networks to generate the flexible and adaptive bipedal gait, which is characteristic of human individuals. This knowledge may help us to understand how the muscle activity during gait is adapted to take different environmental challenges into account. Clinically, more knowledge of the relation between the site and extent of spinal lesions and gait deficits may help us to predict and guide the outcome of interventions.

We have previously shown that reduced transmission in the corticospinal tract (CST) following SCI evaluated by transcranial magnetic stimulation (TMS) and coherence of motor unit activities during gait relate well to reduced function of specific muscles during gait (Barthelemy et al., 2010, 2011, 2013). We have also shown that regional atrophy of the spinal cord at C2 level following SCI is well correlated to specific functional deficits (Lundell et al., 2011a,b). In this study, we aim to integrate these previous data with new data on altered transmission in the vestibulospinal pathway. We hope to demonstrate that combined electrophysiological, imaging, and clinical evaluation of transmission in several descending pathways is necessary to obtain a full understanding of the clinical gait deficits following SCI.

2 METHODS

2.1 PARTICIPANTS

We enrolled 25 individuals with chronic SCI (mean (SD): 43 ± 14 years: 2 female/23 males). The lesions were located at cervical ($n = 20$), thoracic ($n = 4$), or lumbar ($n = 1$) spinal cord levels. Time from injury spanned from 1 to 38 years (mean: 12 years). Description of SCI participants is found in Table 1. The experimental assessments were divided into neuroimaging, electrophysiological, and clinical sessions carried out on different days, but within the timeframe of 1 month. All participants gave their written consent after they received written and oral information about the experiments. We certify that all applicable institutional and

Table 1 Description of the 25 spinal cord injured participants included in this study

Participant number	Male (M)/ female (F)	Age	Years since lesion	Neurologicallevel	AIS	LEMS R (/25)	LEMS L (/25)	BMI	GVS	EMG coherence	Spinal cord imaging
1	M	47	28	C4	D	20	16	24.0	Yes	Yes	Yes
2	M	64	4	C3	D	25	25	22.0	Yes	Yes	Yes
3	M	49	5	L1	D	25	23	24.4	Yes	Yes	Yes
4	M	28	1	T12	D	21	21	19.7	Yes	Yes	Yes
5	M	32	3	T5	D	23	23	17.9	Yes	Yes	Yes
6	F	65	2	C6	D	21	22	26.2	Yes	Yes	Yes
7	M	21	2	C2	D	22	25	21.7	Yes	No coherence	Yes
8	M	44	9	T12	A (D)[a]	15	24	34.7	Yes	Yes	Yes
9	M	43	5	C3	D	22	22	28.4	No	No coherence	Yes
10	M	50	6	C2	D	13	25	27.3	Yes	Yes	Yes
11	M	62	30	C4	D	20	13	22.0	Yes	Yes	Yes
12	M	38	21	C2	D	12	23	23.8	Yes	Yes	Yes
13	M	48	14	C1	D	24	25	22.7	Yes	Yes	Yes
14	M	45	22	C5	D	23	25	24.1	Yes	Yes	Yes
15	M	31	9	C5	D	49	50	22.7	Yes	Yes	No
16	M	62	38	C4	D	22	24	24.7	Yes	Yes	Yes
17	M	32	1	C5	D	25	25	25.8	Yes	Yes	Yes
18	M	54	13	C5	D	23	25	27.7	Yes	Yes	Yes
19	M	45	24	C4	D	43	44	25.7	Yes	Yes	Yes
20	M	42	25	C3	D	43	34	29.0	No	No coherence	Yes
21	M	20	1	C5	D	43	50	24.6	Yes	Yes	No
22	M	49	14	T11	D	50	45	25.7	Yes	Yes	No
23	M	24	1	C2	D	50	48	23.5	No	Yes	No
24	M	28	8	C2	D	50	50	29.5	No	Yes	No
25	F	61	9	C2	D	48	45	22.6	Yes	Yes	No

AIS, American Spinal Injury Association Impairment Scale; LEMS, lower extremity motor score; BMI, body mass index; GVS, assessment with galvanic vestibular stimulation; TMS, assessment with transcranial magnetic stimulation.

[a]*See text for explanation.*

governmental regulations concerning the ethical use of human volunteers were followed during the course of this research. The ethics committee of the Capital Region of Copenhagen approved the study.

2.2 CLINICAL ASSESSMENT

All 25 SCI participants were tested with the following clinical tests. Gait function was assessed clinically by the 6-Min Walking Test (6MWT). In this test, the participant ambulates on a hard flat surface for 6 min and the total distance is recorded (Hedeland et al., 2010). The 6MWT is widely used for the assessment of gait ability in neurological patients and has shown good reliability in the SCI population (Hedeland and Kromann-Andersen, 2009; van Hedel and EMSCI Study Group, 2009). Timed-Up and Go (TUG) is a validated test in which the participant, from sitting in a chair, stands up, walks 3 m at their preferred walking speed, returns to the chair and sits down (van Hedel et al., 2005). Participants were instructed to perform two TUGs, one with a left turn and another one with a right turn. We then took the mean of the time of the two TUGs to determine the mean TUG. The Walking Index for Spinal Cord Injury II (WISCI II) is a validated scale used to assess the amount of physical assistance, braces, or devices required to walk 10 m. This scale is out of 20, where 0 represents a patient unable to stand and 20 represents a person who ambulates with no device, brace, or assistance (Anderson et al., 2008). The Berg Balance Scale (BBS) is a measure of balance that includes 14 tasks that are progressively more challenging for balance. Each task is scored from 0 (unable to perform) to 4 (normal) for a total possible score of 56. It has also been validated for the SCI population (Lemay and Nadeau, 2010; Wirz et al., 2010).

2.3 MAGNETIC RESONANCE IMAGING

Nineteen SCI patients participated in the imaging part of the study. Images were acquired on a Siemens Trio 3 Tesla system using a one-channel birdcage head coil (Siemens Medical Solutions, Erlangen, Germany). A T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE) Sequence (TR = 1540 ms, TE = 3.93 ms, flip angle 9°, sagittal image matrix $192 \times 256 \times 256$, and isometric image resolution 1 mm^3) was used giving a good contrast between the spinal cord and the cerebrospinal fluid (CSF). The image field of view covered the whole brain and the upper cervical spinal cord.

2.4 ELECTROPHYSIOLOGICAL ASSESSMENT

Electromyographic (EMG) activity was recorded bilaterally in Soleus (SOL) and tibialis anterior (TA) muscles. Surface Ag–AgCl disk electrodes were placed over the muscles. The signals were amplified ($\times$1000–5000), band-pass filtered (5–25 to 1000 Hz), then digitized and sampled (2 kHz) to a computer using a micro1401 interface (Spike and Signal software; Cambridge Electronic Design Ltd., Cambridge, UK).

2.4.1 Motor-Evoked Potential and Coherence

Assessment of motor-evoked potentials (MEPs) and coherence was performed in 24 and 23 SCI participants, respectively, on the most impaired leg, as determined by the strength of key muscles in each leg (lower extremity motor score; LEMS) from the International Standards for the Neurological Classification of Spinal Injury (ISNCSCI) (Kirshblum et al., 2011) and confirmed by the patient to be the weakest leg. Fifteen healthy controls (13 men and 2 women; average age: 42 ± 16 years) were also assessed.

For quantitative comparison, TA EMG amplitudes were normalized to the individual participants' maximal compound muscle action potential (M_{max}) elicited by a supramaximal stimulation (1 ms rectangular pulse; 20 mA) of the common peroneal nerve. The participants were sitting with the knee and ankle joints in right angles and stimulation strength was gradually increased until no further increase in the M-waves was observed.

The maximum isometric voluntary contraction (MVC) was also recorded. For this measurement, the participant was sitting in the same position as above. The foot was firmly stabilized on a custom-made pedal, which ensured that all contractions were isometric when dorsiflexion was performed. The participants were asked to perform a maximal isometric dorsiflexion, and online visual feedback of their performance was given by displaying the amplitude of the rectified and integrated (time constant: 200 ms) TA EMG on an oscilloscope placed in front of them. The highest EMG amplitude recorded on either one of those trials was used as MVC in the subsequent experiments.

To assess the connectivity of the CST, we used single-pulse TMS (Magstim Rapid Rate stimulator; Magstim Company Ltd, Dyfed, UK) with the participant sitting as above. A questionnaire was filled out by the participant to make sure that there was no contraindication to the TMS (history of epilepsy, cardiac pacemaker, cranial implants) The stimuli were applied over the leg area of the motor cortex contralateral to the leg tested by using a double-cone coil.

The optimal coil location for evoking an MEP in the TA muscle was found by applying stimuli around the expected hot spot slightly lateral to the vertex, contralateral to the side under investigation. The location with the highest MEP amplitude was marked on a fixed cap. The motor threshold (MT) of the MEP was determined as the stimulus intensity at which three of five stimuli evoked a recognizable MEP, i.e., 100–200 μV during TA contraction equivalent to 10% MVC (Hedelin, 2009). The most impaired side was tested in each participant during the same contraction, and stimulation was applied 5 to 10 times at a stimulation strength of 20% over MT. The amplitude of the MEP in the resting participant and the latency of the MEP during contraction were measured and used for correlation to clinical gait measures.

Coherence was also assessed in the TA of the most impaired leg (two pairs of electrodes; distance between the pairs: ~10 cm; distance between individual electrodes in each pair: 2 cm; recording area: 1 cm^2). Data for analysis of intramuscular coherence were recorded while the participant walked on a treadmill (TechnoGym).

The participant was asked to find a comfortable walking speed that could be sustained over a 5-min recording period. A pressure-sensitive sensor was attached to the heel of the most impaired leg to monitor the time of heel contact during each step cycle. This signal was later used in order to extract EMG during the swing phase of the walking cycle during coherence analysis.

2.4.2 Galvanic Vestibular Stimulation

Galvanic vestibular stimulation (GVS) was used to assess the vestibulospinal tract and was tested on 21 SCI participants and 9 control participants (42 ± 17 years). GVS was applied binaurally, on the mastoid process, using a custom-made constant current stimulator. The cathode was placed behind the right ear and the anode behind the left ear. Electrodes consisted of round metallic plates (8 mm diameter) embedded in electrode cream (Grass EC-2) and secured with adhesive tape. Stimulation consisted of a rectangular 200 ms pulse applied at an intensity of 3–4 mA, depending on the tolerance of each individual. In three individuals, stimulation was below 3 mA (2–2.5 mA).

The individuals were asked to stand on the floor with the feet at the width of the hip and keep their eyes closed. Two acquisitions were recorded: one, while the individuals had their head turned to the left (hence anode backward) and the other one, where individuals had their head turned to the right. To characterize vestibular responses, a series of 40 trials of 5 s each were recorded. GVS was applied in 20 of those trials, and the other 20 trials were used as control to assess background EMG (without stimulation). Stimulated and unstimulated trials were applied in a randomized manner.

2.5 DATA ANALYSIS

2.5.1 Spinal Cord Segmentation

Image correction for gradient nonlinearities was performed off-line using in-house software implemented in Matlab (The MathWorks, Inc., Natick, MA, USA) as described earlier (Lundell et al., 2011a). A midsagital slice was chosen for defining anatomical landmarks (Fig. 1A). A plane perpendicular to the spinal cord was drawn

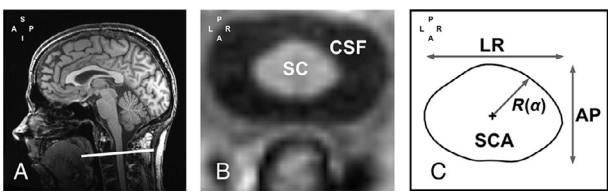

FIGURE 1

MR of spinal cord and area measurements.

Moderated from Lundell et al. (2011a,b) with permission.

from the superior edge of the C2 process and formed the midplane in an interpolated axial dataset with isometric resolution 0.1 mm^3 and 21 slices (one slice is shown in Fig. 1B). The increased resolution was used to account for partial volumes of CSF and spinal cord. Two regions of interest (ROIs) were drawn manually in the midplane covering the spinal cord and the CSF space (Fig. 1B).

The mid-value between the mean intensities in the outer CSF ROI and the inner spinal cord ROI was used as a boundary threshold to outline the spinal cord, and the area of the mask was used as spinal cord area (SCA) (Fig. 1C). To assess the effect of atrophy in oblique directions, the radii from the cord shape center of mass to its border ($R(\alpha)$ were measured over the whole circle with an angular resolution of 6° (Fig. 1C). The spinal cord shape was mirrored for individuals with the largest impairment on the left side such that the $R(\alpha)$ data could be pooled to functional data which was recorded from the most impaired side only. The mean measure of SCA and $R(\alpha)$ from all 21 axial slices was used for the statistics to avoid random effects in single slices from nerve roots, noise, etc. The same observer performed all manual steps blinded to the identity of the individual participants.

2.5.2 Motor-Evoked Potential

The MEP amplitude at rest was measured by taking the peak-to-peak value of each normalized MEP and averaging those. MEP latency was calculated on rectified and averaged MEP traces and corresponded to the delay between the stimulation and the onset of the first significantly higher peak compared to background EMG.

2.5.3 Coherence

Analysis of the networks (e.g., CST) responsible for driving spinal motoneurones during walking can be obtained from coherence and cross-correlation analysis of EMG activity (Barthelemy et al., 2010; Halliday et al., 2003; Hansen et al., 2005; Nielsen et al., 2008). The presynaptic inputs that synchronize the populations of motor units can be estimated by doing a statistical analysis and characterize the frequency content and time course.

The method uses the Neurospec scripts available online (http://www.neurospec.org) and has been described elsewhere (Hansen et al., 2005; Nielsen et al., 2008). Briefly, a Fourier transform-based framework is used for the analysis. This allows that a correlation structure between the paired EMG signals can be characterized as a function of time and frequency (Thorn et al., 2009). Surface EMG was preprocessed using a full-wave rectification. The rectified signals were then assumed to be realizations of stationary zero mean time series, which were denoted by x and y. A periodogram was applied to estimate the power spectra. From these spectra, discrete Fourier transform was constructed from short sections of the data taken at a fixed offset time with respect to a trigger point (in this case the heel strike) in each step cycle. By an average of the periodograms across all step cycles, estimates of the spectra were constructed. For the data presented, a segment length corresponding to the period of TA EMG activity during the swing phase was used. $f_{xx}(\lambda)$ and $f_{yy}(\lambda)$ were used to represent the power spectra of processes x and y, respectively. The

cross-spectrum between x and y was denoted by $f_{xy}(\lambda)$ and was estimated in a similar manner to the auto spectra. The correlation between the EMG signals was assessed through coherence functions in the frequency domain (Halliday et al., 1995). The coherence function between the two rectified EMG signals was defined at frequency λ as:

$$|R_{xy}(\lambda)|^2 = \frac{|f_{xy}(\lambda)|^2}{f_{xx}(\lambda) f_{yy}(\lambda)}$$

On a scale from 0 to 1, coherence functions provide normative measures of linear association.

For the present data, the coherence provides a measure, at each Fourier frequency ë, of the fraction of the activity in one surface EMG signal, which could be predicted by the activity in the second surface EMG signal. In this way, the coherence was used to quantify the strength and frequency of common rhythmic synaptic inputs, which were distributed across the motoneuron pool (Farmer et al., 1993).

Coherence estimates were calculated for paired TA EMG recordings obtained during gait for the 600-ms time period in the swing phase prior to heel strike in control participants. In SCI participants, the TA EMG activity was more variable, and the time period over which coherence was calculated was therefore determined individually in these participants. The amount of coherence was calculated as the area of significant coherence ($p < 0.05$) for each participant within the beta (15–25 Hz) and gamma (35–60 Hz) frequency bands.

2.5.4 Galvanic Vestibular Stimulation

Both the short-latency response (SLR) and medium-latency response (MLR) were analyzed in SOL muscles of both legs (see Fitzpatrick et al., 1994). In two control participants and three SCI participants, gastrocnemius medialis (GM) muscle was analyzed instead of SOL, as responses were clearly seen in GM and not in SOL. EMG in control and stimulated trials were rectified and averaged. Onset of SLR and MLR was determined by superimposing stimulated trace with the unstimulated trace. Facilitatory or inhibitory GVS responses were determined when the EMG activity was above or below a difference of one standard deviation (SD) from the averaged background EMG activity. The latency of the SLR was determined as the beginning of the deflection of the first significant peak above or below background EMG that occurs within 100 ms after the onset of the stimulation. The latency of MLR was determined as the onset of the second significant peak after the onset of the stimulation, or as the first significant peak occurring after 100 ms, if no responses were seen prior to 100 ms. Offset of both SLR and MLR was determined when the stimulated trace no longer differed significantly from the background EMG. Duration of each response was determined by subtracting the time of onset from the time of offset.

Latency and duration of SLR and MLR responses were averaged for both right and left SOL over both conditions (head turned right and turned left). Thus, for each

participant, vestibular transmission is characterized by a mean value for each of the following measures: SLR latency, SLR duration, MLR latency, MLR duration, SLR and MLR amplitude when head is turned left, SLR and MLR amplitude when head is turned right.

2.5.5 Statistics

Statistics was performed using the statistics toolbox in Matlab 7.7.0 (R2008b) (Mathworks Inc., USA). Linear regression was performed to investigate the correlation between variables. Pearson's correlation coefficient was used to report the correlation strength, and relations with $p < 0.05$ were reported as significant.

Best subset regression analysis was used to investigate which of the anatomical and electrophysiological parameters alone or in combination provided the best prediction of the clinical parameters of gait disability using adjusted R^2 in order to take multiple comparisons into account.

For statistical analysis of GVS data, SigmaPlot11 was used. Descriptive statistics was performed, followed by normal data distribution assessment with Kolmogorov–Smirnov test. Student's *t*-test was used to perform group comparisons when distribution of data was normal, otherwise the Mann–Whitney Rank Sum test was used. Values are reported as mean $\pm$ SEM. Statistical significance was reached when $p < 0.05$.

3 RESULTS

The electrophysiological assessments were not carried out in one participant, who was therefore excluded from the MEP and coherence analysis. Paired EMG recordings were not available from two more participants, which precluded coherence analysis.

The MEP and coherence measures have been reported previously (Barthelemy et al., 2010) and have only been summarized in Fig. 2. It is seen that the latency of the TA MEPs was significantly longer (SCI: 40 ± 6 ms; control: 32 ± 2 ms; Rank Sum test $p < 0.001$) and the MEP amplitude significantly lower in the population of SCI participants (SCI: $4 \pm 6\%$Mmax; CTRL: $7 \pm 5\%M_{max}$; $p = 0.04$) as compared to the healthy control population. Significantly lower coherence between the TA EMG recordings was also observed in the SCI participants in both the beta and gamma frequency as compared to the healthy group.

3.1 GALVANIC VESTIBULAR STIMULATION

The muscular responses to galvanic stimulation have not been reported previously and are therefore described in more detail in Figs. 3 and 4. In Fig. 3A, examples of responses evoked by GVS in both conditions (head turned left and head turned right) are illustrated in a control participant. When the head of the participant was turned to the left, GVS induced a short-latency inhibition in SOL muscles

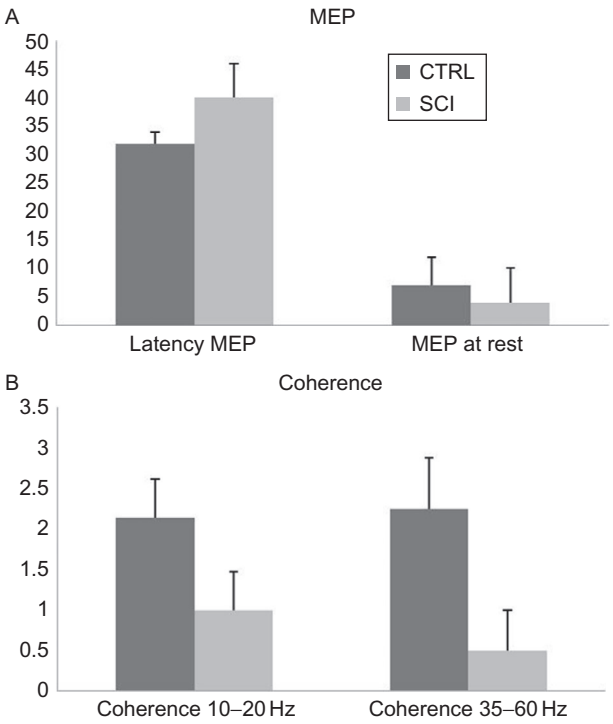

FIGURE 2

Comparison of electrophysiological measurements between control (black) and SCI (gray) group. (A) MEP latency and amplitude at rest are compared between both groups. (B) Coherence in the beta band (10–20 Hz) and gamma band (35–60 Hz) is compared between both groups.

bilaterally (Group data: amplitude: $56 \pm 3\%$; latency: 67 ± 2 ms; duration: 36 ± 4 ms), followed by a medium-latency facilitation (Group data: amplitude: $157 \pm 14\%$; latency: 125 ± 7 ms; duration: 35 ± 7 ms). When the head was turned to the right, responses were less clear and a short-latency facilitation was evoked in SOL (Group data: amplitude: $127 \pm 30\%$; latency: 75 ± 5 ms; duration: 34 ± 8 ms) followed by an inhibitory MLR (amplitude: $69 \pm 13\%$; latency: 138 ± 7 ms; duration: 6 ± 9 ms).

Figure 3B shows data from a SCI participant where the duration of the SLR and MLR were longer than what was seen in controls. Furthermore, the pattern of responses in this participant was different in left and right SOL, whereas both SOL displayed the same response in control participants and in most other SCI participants. For the SCI participant shown in Fig. 3C, no response could be observed in the right leg, but small responses could be observed in the left leg. Thus, there was great variability in the vestibular responses observed in SCI participants.

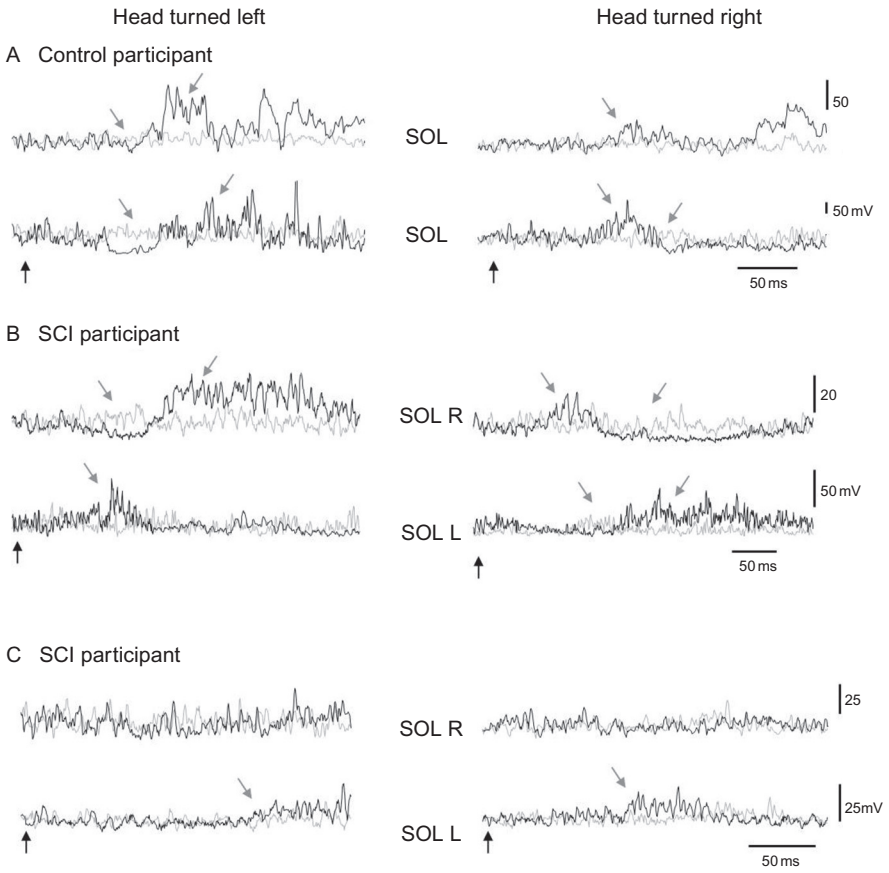

FIGURE 3

Galvanic vestibular stimulation (GVS) responses on raw electromyography (EMG) from a control participant (A) and two spinal cord injured (SCI) participants (B and C) in the standing position, cathode behind right ear, eyes closed and head turned to the left (left column) or to the right (right column). Black traces are averages of 20 stimulated traces and gray traces represent baseline EMG. Black arrows represent the onset of stimulation and the gray arrows the short-latency responses (SLR) and medium-latency responses (MLR). Soleus (SOL) EMG are recorded from the right and left lower limbs.

3.2 CHARACTERIZATION OF VESTIBULAR RESPONSES

Figure 4 shows the quantification of the latency, duration, and amplitude in control and SCI participants. At the intensity of GVS used, SLR and MLR were present in all control participants tested in either one or both SOL. Sixteen out of the 21 SCI participants tested showed SLR and 13 showed an MLR in the SOL. Two participants did not show any responses to GVS. Their data will thus not be taken in consideration in the following section. Average of the SLR and MLR latency in both conditions

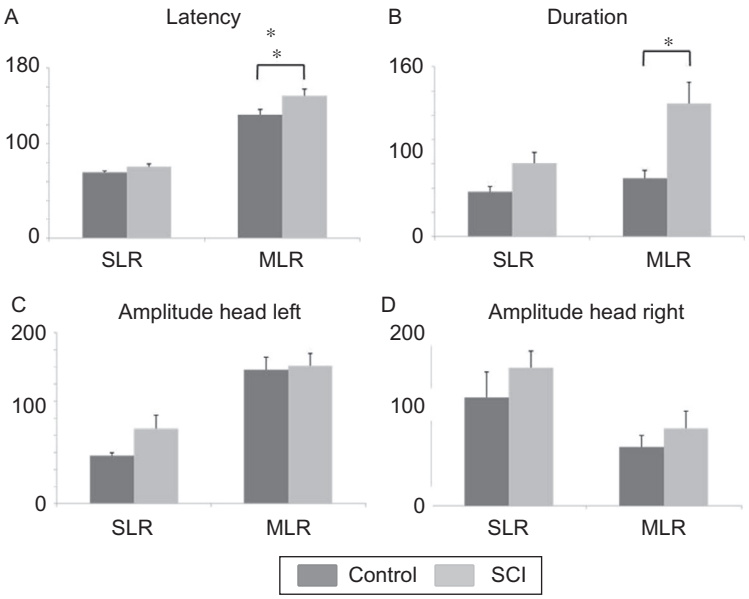

FIGURE 4

Mean latency, duration, and amplitude of short-latency response (SLR) and medium-latency response (MLR) when the head is turned to the left or to the right. The average of the latency and duration has also been made for both conditions (mean). Dark gray bars represent the control group, and light gray bars represent the spinal cord injury (SCI) group $*p<0.05$.

(head left and head right) is shown in Fig. 4A. No statistical difference is observed between the control and SCI groups for the SLR (69 ± 2 ms for controls and 75 ± 3 ms for SCI; $p=0.152$), but the difference was significant for the MLR (131 ± 6 ms for controls and 153 ± 7 ms for SCI; $p=0.02$).

Differences were also observed in the duration of the vestibular responses. Figure 4B illustrates that the duration of both SLR and MLR were longer in the SCI group compared to the control group, but only reached significance for MLR response (SLR: 37 ± 4 ms for controls, 56 ± 11 ms for SCI, $p=0.48$; MLR: 48 ± 7 ms for controls, 123 ± 30 ms SCI, $p=0.038$).

The amplitude of vestibular responses of SCI participants was not significantly different from the control participants, whether the head was turned to the left or to the right (see Fig. 4C and D). However, a tendency was observed where the inhibition occurring in SOL in the SLR during head left condition and in the MLR during head right condition was decreased in SCI compared to controls (amplitude of inhibitory SLR head left: $56\pm3\%$ of background EMG for controls and $88\pm15\%$ for SCI; amplitude of inhibitory MLR head right: $69\pm13\%$ for controls and $91\pm20\%$ for SCI; $p>0.05$ for both conditions).

3.3 WHICH ELECTROPHYSIOLOGICAL AND ANATOMICAL PARAMETERS PROVIDE THE BEST PREDICTION OF GAIT AND BALANCE FUNCTION?

Initially, Pearson product correlation was used to determine which of the MEP parameters (latency or amplitude), coherence parameters (beta and gamma coherence), and galvanic stimulation parameters (SLR and MLR latency and duration) provided the best correlation with the clinical measures of gait function. These correlations are summarized in Table 2. It can be seen that MEP amplitude provided a better correlation than MEP latency in all cases (bold values), that beta coherence provided a better correlation than gamma coherence except in the case of WISCI, and that MLR latency and duration provided better correlation than SLR latency and duration.

Table 2 Pearson product correlation coefficients are shown for different combinations of electrophysiological, anatomical, and clinical parameters

	6-Min Walk Test	Timed-Up and Go	Walking Index Spinal Cord Injury	Gait speed	Berg Balance Scale
MEP latency	−0.361 (0.13)	0.256 (0.3)	−0.298 (0.22)	−0.440 (0.07)	−0.462 (0.05)
MEP amplitude	**0.584 (0.02)**	**−0.417 (0.12)**	**0.572 (0.03)**	**0.496 (0.07)**	**0.492 (0.06)**
Coherence beta	**0.375 (0.07)**	**−0.319 (0.14)**	0.185 (0.4)	**0.450 (0.03)**	**0.526 (0.01)**
Coherence gamma	0.355 (0.1)	−0.285 (0.19)	**0.199 (0.36)**	0.393 (0.07)	0.473 (0.02)
SLR latency	−0.389 (0.12)	−0.00834 (0.9)	0.0384 (0.88)	0.114 (0.66)	0.175 (0.5)
SLR duration	0.168 (0.52)	0.278 (0.3)	−0.108 (0.7)	−0.437 (0.09)	−0.681 (0.003)
MLR latency	−0.475 (0.06)	**0.551 (0.03)**	−0.368 (0.16)	**−0.493 (0.05)**	−0.460 (0.07)
MLR duration	**−0.479 (0.06)**	0.341 (0.2)	**−0.408 (0.11)**	−0.439 (0.09)	**−0.816 (0.0001)**
SCA	**0.548 (0.02)**	−0.298 (0.23)	**0.261 (0.3)**	**0.588 (0.01)**	**0.588 (0.01)**
LR	0.465 (0.052)	**−0.452 (0.06)**	0.209 (0.4)	0.553 (0.02)	0.242 (0.33)
AP	0.231 (0.36)	0.0707 (0.8)	0.093 (0.7)	0.297 (0.25)	0.405 (0.1)

p *Values are given in parentheses following the correlation values. The electrophysiological measures were divided into categories related to transcranial magnetic stimulation, coherence, and vestibular stimulation. These have been separated in the table by bold lines. Within each category, the combination of parameters with the highest correlation coefficient was chosen for further analysis. These have been marked with bold in the table. MEP, motor-evoked potential; SLR, short-latency response following galvanic stimulation; MLR, medium-latency response following galvanic stimulation; SCA, spinal cord area; LR, left–right diameter of spinal cord; AP, anterior–posterior diameter of spinal cord.*

Interestingly, while GVS responses were mostly related to functional balance tests (BBS and TUG) rather than gait (speed), TMS measures were better correlated to gait tests (6-min test and gait speed) rather than balance tests. Coherence measures mainly in the Beta band were correlated to both balance (BBS) and gait speed. No significant correlation was found between the measures of vestibulospinal (SLR and MLR latency or duration) and corticospinal transmission (MEP amplitude and latency or beta or gamma coherence). However, balance and gait tests were strongly correlated to each other ($r = 0.799$, $p < 0.001$).

Among the anatomical measures, SCA generally provided better correlation than the more specific left–right (LR) and anterioposterior (AP) measures, except in the case of TUG, where LR provided a better correlation. LR also provided a better correlation in all other cases than AP.

In the subsequent analysis, the measures providing the best correlation (marked in bold in Table 2) were used.

Best subset regression showed that the MEP amplitude was the best single predictor of 6MWT and WISCI (r^2 values of 0.34–0.38), whereas TUG, gait speed, and the BBS were best predicted by either the MLR latency or duration (r^2 values of 0.4–0.77). Adding one or more measures only improved the prediction of clinical function significantly in the case of WISCI, where a combination of MEP amplitude, gamma coherence, and MLR latency reached an adjusted r^2 value of 0.67 as compared to r^2 values less than 0.35 for the individual parameters. In the other cases, adding more parameters only moderately improved the prediction, and in the case of the BBS yielded a smaller adjusted R^2 value.

3.4 CORRELATION OF DIRECTIONAL-SPECIFIC SPINAL CORD ATROPHY AND ELECTROPHYSIOLOGICAL AND CLINICAL MEASURES

Figure 5 shows the radial correlations between spinal cord radius and the electrophysiological and clinical measures. The main regions affected by atrophy in relation to the specific functional assessments are schematically shown as red (gray in the print version) sectors in the spinal cord illustrations.

Radial correlations to MEP amplitude were found in the lateral–ventral quadrant on the most impaired side (Fig. 5A). MEP latency did not correlate significantly to changes in radius.

Coherence in the beta and gamma bands were best correlated to the total spinal cord atrophy ($r^2 = 0.31$). Radii in bilateral sectors in the dorsolateral direction were found to correlate significantly with the measures of beta and gamma coherence. Similar structures were seen for both frequency bands. In general, correlations were stronger on the side ipsilateral to the functionally most impaired leg.

Neither of the GVS measures of SLR and MLR latency and duration were correlated to any of the measures of spinal cord atrophy.

TUG and WISCI also showed no significant correlation with any of the measures of spinal cord atrophy. 6MWT and gait speed showed significant correlation with the LR measure of spinal cord atrophy (correlation coefficients of 0.47 and 0.55, respectively),

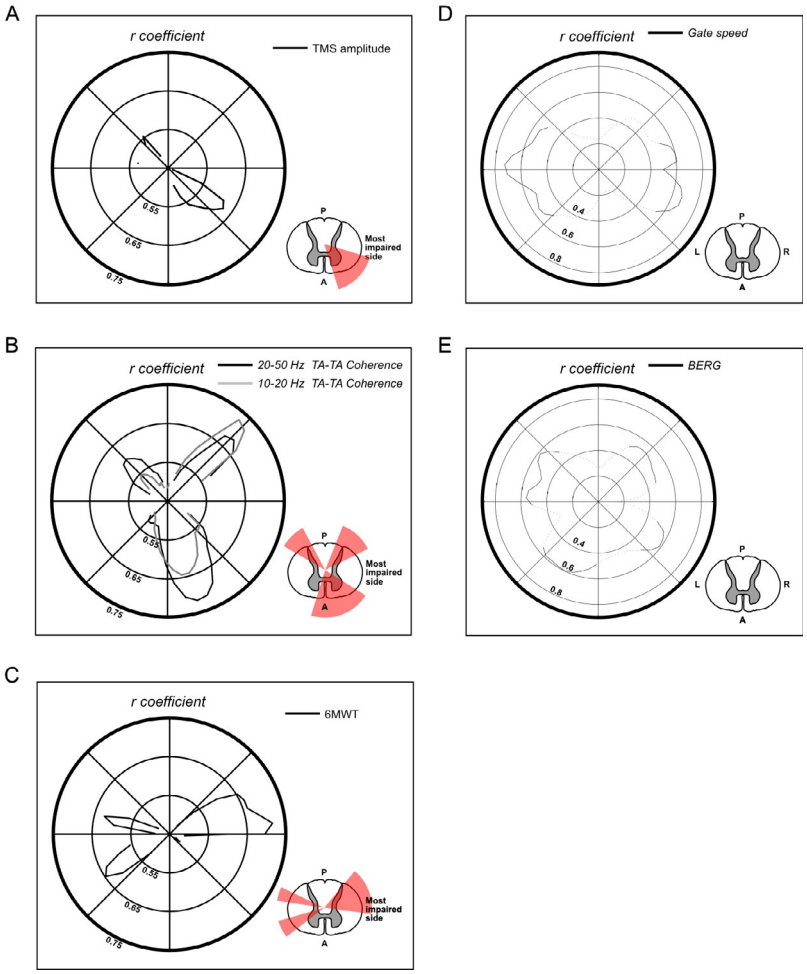

FIGURE 5

Distribution of significant correlations between spinal cord area and electrophysiological and clinical measures.

whereas no significant correlation was found for the AP measure (correlation coefficients of 0.23 and 0.29, respectively). In line with this, radial correlations with both 6MWT and gait speed were found bilaterally in the lateral columns (Fig. 5C and D).

The BBS was correlated significantly only with the total SCA, whereas no correlation was found with either the LR or the AP measure.

Narrow significant radial correlations were found ventro- and dorsolaterally on both sides of the spinal cord (Fig. 5E).

No significant correlation was found between the measures of vestibulospinal (SLR and MLR latency or duration) and corticospinal transmission (MEP amplitude and latency or beta or gamma coherence).

4 DISCUSSION

This study has demonstrated that measures of corticospinal and vestibulospinal transmission in combination are moderately good predictors of gait and balance function following SCI. The study has further shown that the electrophysiological measures (MEP amplitude and coherence) as expected are related to atrophy of the parts of the spinal cord where the CST is located. Gait and balance function on the other hand appear to be related to atrophy of wider areas of the spinal cord supporting that lesion of several different descending pathways contribute to the clinical gait deficit following SCI.

The anatomical and electrophysiological measures generally yielded correlation coefficients with the clinical measures of gait function that were only moderately good. This may be related to simple variability and uncertainty of the methods used for obtaining the different measures, but it may also be related to nonneurological factors that may contribute to reduced gait function (arthritis, cardiovascular function, etc.). In addition, each of the anatomical and electrophysiological measures is likely to provide only partial information of the functional transmission in the pathways that are involved in the control of gait. The anatomical measures provide a quantitative measure of atrophy in the spinal cord, which in the case of direction-specific atrophy measures may be related to some extent to lesion of specific pathways, but they do not provide any information regarding transmission in the different pathways. Functional transmission in a fraction of surviving descending fibers may be sufficient to preserve a relatively good gait function and severely impaired transmission may be seen despite limited atrophy in the spinal cord.

It has recently been reported for healthy controls that diffusion anisotropy in the lateral columns detected with diffusion tensor imaging (DTI), an MRI technique sensitive to water mobility on the micrometer scale, is related to performance in a dexterous precision task where a large CST contribution was expected (Lindberg et al., 2010). Diffusion anisotropy may indicate microstructural features like axonal myelination, packing, radii, curvature and angular distribution. However, anisotropy is not independent of tract size, which will induce partial volumes of isotropic CSF and gray matter (Voss and Schiff, 2009). In general, we observed stronger effects of atrophy on the most impaired side, but we should mention that the use of the mask center of mass as spinal cord center is ambiguous, as it will be shifted itself following atrophy. This factor decreases the sensitivity and may induce false positives in the antiparallel direction. This could in our case for instance be the dorsolateral sector contralateral to the most impaired leg in Fig. 5. Model-based segmentation could potentially introduce more detailed tract information (Ellingson et al., 2008) or decomposition of spinal cord shape into modes of change using sparse principal component

analysis as previously applied to the corpus callosum (Ryberg et al., 2008). For a more specific differentiation between different levels of the spinal cord, multiple levels could be analyzed from individual slices or a connected volume of the spinal cord. Methods for monitoring the progression of axonal degeneration from a lesion in the subacute stage could have clinical interest. DTI has also been suggested for this, but no sensitivity to tract specific functions has yet been presented. The more demanding technical challenges for spinal cord DTI should be compared to the simplicity and robustness of the acquisition and the analysis used in this study.

MEP latency, which is used frequently in the clinic for evaluation of central conduction time, was found in this study to be of little relevance for gait function. This is not surprising since the latency of the MEP rests solely on transmission in the fastest conducting corticospinal fibers. These fibers are a very small minority in the CST and preserved or impaired transmission in them may not provide much information about the functional contribution to gait of the remaining part of the tract. Furthermore, differences in transmission time in peripheral fibers were not taken into account in this study and may also contribute to the low correlation values for the MEP latency. The MEP amplitude at rest, which showed much better correlation with gait function, evaluates the ability of the descending volley in a range of corticospinal fibers to discharge the spinal motoneurones and may therefore provide a more relevant and sensitive measure of corticospinal transmission. It should be noted in this relation that all amplitude measures in the present study were normalized to the maximal M response in the muscle in order to take variability in skin and tissue resistance and electrode placement in the individuals into account. This normalization is essential from a theoretical point of view and was also shown as part of this study to be essential in order to provide sufficiently reliable data that could be correlated to gait function. Despite this, normalization to the maximal M response is rarely done in clinical studies.

Coherence estimates of coupled beta (15–25 Hz) and gamma (35–60 Hz) activity in populations of TA motor units recorded in the swing phase during gait have been shown previously to be well correlated to foot drop in SCI individuals (Barthelemy et al., 2010) and were in this study found to be as well correlated to clinical parameters of gait and balance function as the MEP amplitude. Gamma band coherence of TA EMG activity has also been found to correlate to the development of a more precise and consistent gait pattern in children in the age group 6–12 years (Petersen et al., 2010). Reduced gamma band coherence also correlates to impaired toe lift and heel strike in children with cerebral palsy (Petersen et al., 2013). TA EMG coherence thus provides a clinically relevant electrophysiological measure, which is furthermore easy to apply in a clinical setting with little requirement of technical equipment. Calculation of TA EMG coherence only requires recording of EMG activity from two pairs of electrodes placed at a sufficiently long distance from each other (i.e., >6 cm; cf. Halliday et al., 2003) during approximately 5 min of gait.

Previous studies have strongly implicated that transmission in the CST is responsible for the occurrence of TA EMG coherence in the beta and gamma bands. Coherence in similar frequency bands is thus observed between electroencephalography recorded

from the leg area of the motor cortex and the TA muscle both during static contraction and during gait (Petersen et al., 2012). Furthermore, beta and gamma coherence are selectively reduced in patients with lesion of the CST whether caused by stroke (Nielsen et al., 2008), SCI (Hansen et al., 2005) or cerebral palsy (Petersen et al., 2013). Finally, TMS of the motor cortex may induce oscillations at the relevant frequencies in the TA muscle (Hansen and Nielsen, 2004). In this study, the observation that coherence in the beta and gamma bands correlated to atrophy in the dorsal part of the lateral columns is well in-line with these previous findings and further strengthens the idea that the CST is involved in generating oscillations of the muscle activity in the beta and gamma frequency bands. This does not exclude the possibility that other mechanisms such as sensory feedback contribute to the strength of coherence as has been suggested (Witham et al., 2011), but it should be noted that no correlation was found with atrophy of the dorsal columns where the major sensory pathways from touch and proprioceptive receptors are located.

In this study, changes were observed in MLR response, but no significant changes were observed in the SLR response. This observation may suggest that the responses are mediated by different neuronal networks as suggested by others (Britton et al., 1993; Cathers et al., 2005; Muise et al., 2012).

The duration or amplitude of the MLR responses to GVS was shown to be strongly correlated to the BBS, whereas the correlation with clinical measures of gait function was generally only moderate. This is not surprising given the direct involvement of the vestibulospinal tract in balance control, whereas the role of balance for gait function is somewhat more indirect. Nevertheless, it should be noted that the MLR response provided as good measures of gait function as the MEP and coherence measures and that BBS was found to be well correlated to gait speed. This is consistent with the idea that impaired balance to some extent affects the ability to walk fast.

SLR did not correlate as well with BBS, although a clear tendency for increased SLR duration in the most impaired participants was observed. This is not surprising as many recent studies have suggested that SLR does not reflect vestibular function to the same extent as MLR (Britton et al., 1993; Cathers et al., 2005; Muise et al., 2012). Our findings thus strongly suggest that MLR rather than SLR provides information of vestibulospinal transmission that is relevant for both balance and gait function following SCI.

Importantly, vestibulospinal transmission did not correlate to transmission in CST measured by TMS or coherence in the beta and gamma band. Either of those measures was correlated to specific functional tests and suggests that both GVS and TMS are correlated to specific functions and in that sense are complementary investigation tools to try and determine the pathways that remain after an incomplete SCI and more importantly the function that might be impaired.

We decided to analyze the mean of duration and amplitude of SLR and MLR responses in both legs similar to Iles et al. (2004), although the summation of the output from the left and right vestibular organs has been shown to be nonlinear (Day et al., 2010), and although responses obtained with the head turned to

the left (putting the anode ipsilaterally to the SOL) seem to show the most significant difference between control and SCI groups (Liechti et al., 2008). The reason for this is that when taking single individual data for correlations it was clear that responses were not only dependent on the side the head was turned but also on the side that was the most affected by the lesion in each individual. Indeed in some SCI participants (e.g., Fig. 2C), one side did not show any response to the GVS, regardless of the direction of the head, but the other side showed responses. These unilateral responses might be enough to sustain some balance capacities in the participant. Thus, the responses were averaged in both legs in order to assess the total vestibular output in each SCI participant and compare it to control values.

5 CONCLUSION

Gait and balance function appear to be related to impaired transmission in several different descending pathways, which will need to be taken into account when considering possible gait and balance interventions. Furthermore, more studies are needed to improve sensitivity of electrophysiological and imaging measurements for the SCI population.

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