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PREPARATORY CORTICAL AND SPINAL SETTINGS TO COUNTERACT ANTICIPATED AND NON-ANTICIPATED PERTURBATIONS

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Abstract—Little is known about how the central nervous system prepares postural responses differently in anticipated compared to non-anticipated perturbations. To investigate this, participants were exposed to translational and rotational perturbations presented in a blocked (anticipated) and a random (non-anticipated) design. The preparatory setting ('central set') was measured by H-reflexes, motor-evoked potentials (MEPs), and short-interval intracortical inhibition (SICI) shortly *before* perturbation onset in the soleus of 15 healthy adults. Additionally, the behavioral consequences of differential preparatory settings were analyzed by comparing the short- (SLR), medium- (MLR), and long-latency response (LLR) of the soleus *after* anticipated and non-anticipated rotations and translations. H-reflexes elicited *before* perturbation were different between conditions ($p = 0.023$) with larger amplitudes in anticipated translations compared to anticipated rotations (37.0%; $p = 0.048$). Reduced SICI was found in the three conditions containing perturbations compared to static standing ($p < 0.001$). Muscular responses assessed *after* perturbations remained unchanged for the SLR and MLR, whereas the LLR was decreased in anticipated rotations (−36.2%; $p = 0.002$) and increased in anticipated translations (16.7%; $p = 0.046$) compared to the corresponding non-anticipated perturbation. As the SLR and MLR are organized at the spinal and the LLR at the cortical level, the preparatory setting seems to mainly influence cortically mediated postural responses. However, the modulation of the H-reflex *before* anticipated perturbations indicates that supraspinal centers adjusted Ia-afferent transmission for the soleus in a perturbation-specific manner. Intracortical inhibition was also modulated but differentiates to a lesser extent only between perturbation conditions and unperturbed stance. **© 2017 The Authors. Published by Elsevier Ltd on behalf of IBRO. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).**

Key words: central set, H-reflex, MEP, postural disturbance, short-interval intracortical inhibition, SICI.

INTRODUCTION

Muscular responses to postural perturbations are regulated at the spinal and supraspinal levels. The earliest muscular response, called the short-latency response (SLR), is elicited by a monosynaptic spinal circuit (Matthews, 1991; Corden et al., 2000). The SLR is followed by the medium-latency response (MLR) that is also assumed to be spinally modulated and evoked by group II-afferent fibers (Nardone and Schieppati, 1998; Grey et al., 2001; Uysal et al., 2009). The subsequent long-latency response (LLR) is mediated by supraspinal centers (Beloozerova et al., 2003; Taube et al., 2006). Not only does the involvement of supraspinal centers increase with the time of postural response progression (Taube et al., 2006), contribution from these supraspinal regions becomes greater when an individual can anticipate the characteristics of the upcoming perturbation (Jacobs and Horak, 2007).

It was previously shown that anticipation alters the behavioral outcomes of balance recovery. In this regard it was demonstrated that (a) when individuals are able to practice responding to a set of the same backward translation perturbations, they reduce postural sway, (b) individuals will over- or under-react when they unexpectedly experience a smaller or larger perturbation amplitude, respectively, than anticipated, and (c) the stretch response in the agonist is selectively tuned to the perturbation amplitude when knowledge and prior experience about the perturbation is available but remains unaltered when perturbation amplitudes are unknown and randomized (Horak et al., 1989). These observations indicate that postural responses to a perturbation are not only related to sensory reactions but they also depend on prior experience and/or knowledge of the upcoming perturbation. This preparatory neuromotor state based on the initial context has been termed as 'central set' (Horak et al., 1989; Prochazka, 1989; Jacobs and Horak, 2007). However, as the term central set might be accidentally referred to only supraspinal structures, we have chosen the term 'preparatory setting'

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Abbreviations: EEG, electroencephalogram; EMG, electromyography; LLR, long-latency response; mANG, maximal angle movement; MEPs, motor-evoked potentials; MLR, medium-latency response; PNS, peripheral nerve stimulation; RMS, root mean square; SICI, short-interval intracortical inhibition; SLR, short-latency response; TMS, transcranial magnetic stimulation.

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in the present study to clearly incorporate spinal and supraspinal structures.

A limitation of these classical behavioral postural measures is that they only capture the consequences of this preparatory setting (Bolton, 2015). In contrast, neurophysiological measurements are required to investigate how preparatory setting is achieved in anticipation of an upcoming perturbation. Changes in cortical activation assessed by electroencephalogram (EEG) prior to a perturbation indicate that the preparatory setting is – at least partly – cortically mediated. This is evidenced by EEG responses depending on pre-cues about the occurrence of a perturbation (Jacobs et al., 2008) as well as by higher preparatory cortical EEG-signals in non-anticipated compared to anticipated perturbations (Mochizuki et al., 2010).

To extend our knowledge on how the central nervous system prepares postural reactions in anticipation of a loss of balance, an experimental paradigm similar to that initially introduced by Nashner (1976) was used. Two types of perturbations, toe-up support surface rotations and backward translations, were presented with predictability (i.e., blocked order), such that the individual could anticipate the upcoming type of perturbation, or without predictability (i.e., random order), such that the individual could not anticipate what perturbation type was occurring next. While the calf muscles are stretched similarly for both types of perturbations, the response of the calf muscles to a rotational movement is counterproductive as it further accelerates a backward fall. In contrast, calf muscle activity in response to a translational movement helps to re-establish posture. Thus, although the stretch stimulus to the muscle is the same, the muscular reaction should be contrariwise in order to efficiently stabilize posture.

To assess anticipatory strategies of the central nervous system to these perturbations, the present study applied peripheral nerve stimulation (PNS) and transcranial magnetic stimulation (TMS) shortly before the perturbation to monitor activity at the spinal and motor cortical levels. Specifically, PNS was used to elicit Hoffmann's reflex (H-reflex) responses in order to infer anticipatory changes in Ia-afferent transmission at the spinal level, while TMS was applied to assess short-interval intracortical inhibition (SICI). Previous studies have shown that the SICI technique provides information about the level of $GABA_A$-ergic intracortical inhibition (Ziemann et al., 1996; Chen, 2004; Di Lazzaro et al., 2006), which seems essential for an adequate motor control (Soto et al., 2006; Sidhu et al., 2013). Based on previous studies that have indicated reduced SICI with increased postural task difficulty (Papegaaij et al., 2016a), we hypothesized that a decreased SICI would be observed in the perturbation conditions compared to unperturbed stance and greater SICI reductions in non-anticipated compared to anticipated perturbations. At the spinal level, it was shown that the H-reflex is task-specifically modulated before movement initiation (Leukel et al., 2008; Petersen et al., 2009). Therefore, it was hypothesized that the H-reflex would adapt in a perturbation-specific manner before the onset of the anticipated perturbation, i.e., decreased H-reflex amplitudes before rotation compared to translation. Finally, muscular response patterns after the perturbation were analyzed in order to assess the consequences of these anticipatory strategies to the subsequent behavioral measures. In accordance with previous observations (Nashner, 1976), we hypothesized that muscular responses would be modulated in a similar manner as the H-reflexes, i.e., facilitation after anticipated translations and inhibition after anticipated rotations when compared to the corresponding non-anticipated perturbation.

EXPERIMENTAL PROCEDURES

Participants

Fifteen adults (27.7 ± 3.5 years, 1.75 ± 0.11 m, 68.9 ± 12.7 kg; five female and 10 male) without any neurological or orthopedic injuries participated in this study. Prior to the experiment, all participants were thoroughly informed and gave written consent to the experimental procedure. This work was accepted by the local ethics committee and respected the latest ethical principles of the Declaration of Helsinki.

Experimental procedure

Participants were exposed to four different experimental conditions in a randomized order: (1) blocked Rotation (ROT), (2) blocked Translation (TRA), (3) Random involving ROT and TRA (RAN), (4) Static upright stance without any perturbations (STA). The preparatory setting was therefore manipulated by the type of perturbation (i.e., perturbation-specific; ROT or TRA) and its predictability (anticipated or non-anticipated). Specifically, participants could anticipate the type of perturbation in the blocked design (i.e., ROT and TRA) since all trials within a block consisted of the same perturbation, whereas they could not anticipate the perturbation type in the RAN condition because rotational and translational perturbations were randomly presented. Examining the predictability of perturbation onset was not the focus of this study and thus, the onset of the perturbation could always be anticipated due to a preceding acoustic cue occurring a constant 1000 ms prior to perturbation onset (Fig. 1). The average amplitude for the toe-up rotation was 9.23° and lasted 130 ms with an acceleration of 2700°/s^2 and a maximal velocity of 97°/s. Translational perturbations were induced by moving the support surface 0.01 m backwards in 270 ms with an acceleration of 5.49 m/s^2 and a maximal velocity of 0.74 m/s. Thus, the surface translations and rotations were faster than in previous studies investigating the effect of anticipatory settings to counteract perturbations (Horak et al., 1989: translation velocity of 0.15 m/s; Nashner, 1976: translations provoked an ankle angle rotation of 0.5°/s, rotation velocity of 6°/s). Due to security reasons, a hand rail was positioned next to the participants. In case of a loss of balance, participants had the possibility to stabilize posture by grasping the hand rail. However, this was never the case.

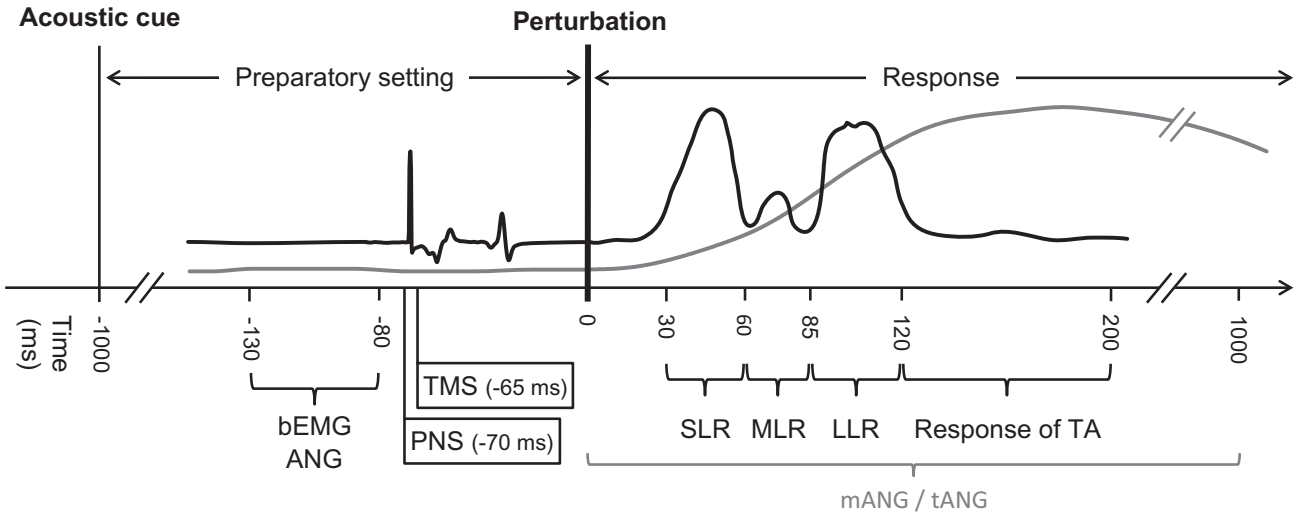

Fig. 1. Experimental procedure. Black line is representing an exemplary EMG signal of m. soleus. Gray line is representing an exemplary ankle angle. bEMG: Background EMG of SOL and m. tibialis anterior (TA) before the stimulations. SLR: Short-latency response of SOL after trials without stimulations (CON). MLR: Medium-latency response of SOL after CON trials. LLR: Long-latency response of SOL after CON trials. Response of TA: Response of TA after CON trials. ANG: Angle of the ankle joint before stimulations. mANG: Maximal amplitude of the ankle joint angle after CON trials. tANG: Time point of maximal amplitude of the ankle joint angle after CON trials. TMS: Transcranial magnetic stimulation (single and double pulse stimulations). PNS: Peripheral nerve stimulation.

For each trial, participants stood in a natural upright position with arms akimbo on a custom-made perturbation machine. The joint center of the participant's ankle was aligned with the center of rotation of the support surface pedals. After every 8 s, participants received an acoustic cue to warn them that the next perturbation would occur in 1000 ms. In response to each perturbation, participants were required to recover their balance as quickly as possible without stepping. At the beginning of the experiment, participants were given five repetitions of each ROT and TRA for familiarization purposes and data from these trials were not analyzed.

The influence of the preparatory setting on neuromuscular responses was investigated *before* and *after* the perturbation (see Fig. 1). H-reflexes, motor-evoked potentials (MEPs) and SICI were elicited by PNS and TMS, respectively, to occur around 35 ms before perturbation onset. This ensured that the evoked responses were not affected by the onset of perturbation. Therefore, PNS was applied 70 ms and TMS 65 ms before the start of the perturbation (as the latency of the H-reflex is several milliseconds longer than the latency of the MEP). In some trials, no stimulation was applied (i.e., CON). Each stimulation type was administered 16 times in a randomized order resulting in 16 H-reflex, 16 MEP, and 16 SICI, responses per condition. In addition to these trials, 16 CON trials were collected and thus, participants experienced 64 trials for each experimental condition (total duration of one condition: 9 min). The exception was for the STA condition, where CON was not implemented since no perturbations were administered. As a result, only 48 trials were collected for the STA condition (total duration: 7 min). All participants were provided a 2-min rest between conditions and a 10-min rest period halfway through the experiment (total duration of the experiment: 48 min).

Electromyography (EMG)

Muscular activity in response to stimulations and perturbations was assessed with surface EMG recordings from m. soleus (SOL) and m. tibialis anterior (TA). EMG data were recorded on the right leg with a custom-built EMG system (EISA, University of Freiburg, Germany). Electrodes (34 mm, Ag/AgCl, Ambu Blue Sensor P, Ballerup, Denmark) were placed according to SENIAM guidelines (Hermens et al., 2000). Interelectrode impedance was lowered by shaving, degreasing, and lightly abrading the skin. A velcro-strap was fixed around the lower leg and served as reference electrode. EMG data were amplified (×1000), bandpass filtered (10–1000 Hz), and recorded at 4 kHz with custom-built software (Imago Record, Pfitec, Endingen, Germany).

The SOL EMG recording was also used to provide participants with online feedback while they were standing and awaiting the perturbations. For this purpose, mean and standard deviation of SOL activity during static stance was assessed during a 5-s period at the beginning of the experiment. Throughout the experiment, two horizontal lines representing the calculated static stance mean ± 2 standard deviation EMG activity were displayed on a screen along with feedback about the ongoing muscle activity. Participants were instructed to keep their current muscle activity within the two horizontal lines. This was to ensure that background muscular activation and thus, postural position, remained similar throughout the experiment. In pilot experiments, feedback of current EMG activity was found to be more effective than feedback of the ankle

angle, as measured by a goniometer, to ensure both a consistent postural position and muscle activity.

Ankle angle recording

The ankle joint angle was measured with an electro-goniometer (MP20, Megatron Elektronik, Putzbrunn, Germany). The center of rotation was placed over the ankle joint axis and the two endplates were tightly fixed on the foot and shank, respectively. Goniometer data was recorded at 4 kHz with Imago Record. The ankle angle change (dorsiflexion) is expressed as deviation from the natural standing position.

Transcranial magnetic stimulation

A butterfly coil (D-B80, MagVenture A/S, Farum, Denmark; Ø 95 mm, 120° angle) connected to a transcranial magnetic stimulator (MagPro X100 with MagOption, MagVenture A/S, Farum, Denmark) was used. Biphasic waveform pulses were applied 65 ms before perturbation onset to elicit MEPs in the right SOL. The coil was oriented with the handle backward and was moved systematically over the left motor cortex to identify the hotspot for the right SOL. At this position, the coil was fixed to the head with a custom-built helmet which allowed the participants to stand freely. The helmet inclusive coil was fixed to the ceiling by an elastic cord to reduce the weight on participants' head. A posterior–anterior current flow in the interhemispheric fissure was induced with TMS. Motor threshold was identified as the lowest stimulation intensity that elicited an MEP of 50 μV in at least three of five consecutive trials during upright stance. 120% of MT was used for the control MEPs (single pulses). Double-pulse stimulation with a time interval of 2.5 ms between pulses was applied to identify SICI. The first pulse was set at 80% and the second pulse at 120% of the motor threshold. Intensities for single and double pulses were kept constant throughout the experiment.

H-reflex

The tibial nerve was electrically stimulated (PNS; square-wave pulse of 1 ms; Digitimer DS7A, Digitimer Ltd, Hertfordshire, UK) 70 ms before perturbation onset to evoke H-reflexes in the right SOL. For this purpose the cathode (2-cm diameter) was fixed over the nerve in the popliteal fossa and the anode (4 × 4 cm) was positioned below the patella. An H-reflex recruitment curve was recorded during upright stance. The stimulation intensity for the experiment was then set to evoke H-reflexes with a size of 50% of the maximal H-reflex and was kept constant throughout the experiment. This stimulation intensity was chosen to provide a corresponding M-wave to help ensure stimulus constancy (Zehr, 2002) and to ensure that the H-reflex was located on the ascending part of the recruitment curve (Grospretre and Martin, 2012) .

Data processing

Muscular EMG and angular data were analyzed offline with MatLab (Version 2014b; The MathWorks, Inc., Natick, MA). Background EMG (bEMG) was determined by calculating the root mean square (RMS) value over a 50-ms time window prior to PNS or TMS stimulation and was equivalent to 80–130 ms before perturbation onset. In this same time window, the background ankle angle (ANG) was calculated as the mean angle of the ankle joint. The bEMG and ANG for the same time windows were analyzed for the CON trials (i.e., trials without stimulation).

SOL H-reflexes as well as SOL and TA MEPs and SICI were analyzed by calculating the peak-to-peak amplitudes in the appropriate time frame (approximately 40–10 ms before perturbation onset). The specific time frame was individually set for each participant. Short-interval intracortical inhibition was expressed as the percentage difference between the mean peak-to-peak values of single- and double-pulse stimulations for each experimental condition.

As both PNS and TMS elicited muscular contractions that led to postural movements, the EMG activity *after* a perturbation were determined from the CON trials, when no stimuli were presented. For these trials, the RMS of the SOL EMG signal for three perturbation-evoked responses were calculated based on their latencies. The SLR was set from 30 to 60 ms after perturbation onset (Rinalduzzi et al., 2015), the MLR from 60 to 85 ms (Taube et al., 2006) and the LLR from 85 to 120 ms (Taube et al., 2006). The TA EMG response (RMS) was assessed from 120 to 200 ms after perturbation onset (i.e., after the LLR in SOL) because the antagonistic TA serves to stabilize the initial postural response. To assess postural responses in the ankle joint, the maximal angle movement (mANG) and the time of mANG (tANG) were calculated within a time window of 1000 ms after perturbation onset.

When analyzing the preparatory setting *before* the perturbation in the RAN condition, the perturbation type was not taken into account and thus, the analysis did not differentiate between the preparatory setting for random rotations (RAN_ROT) and for random translations (RAN_TRA). This was because participants could not anticipate the upcoming perturbation type and differentially prepare for RAN_ROT and RAN_TRA. In contrast, when analyzing the muscular responses *after* the onset of perturbation, there was the need for differentiating between RAN_ROT and RAN_TRA as the muscular responses greatly differed between rotations and translations. This separation enabled us to compare the response pattern in these non-anticipated perturbations (i.e., RAN_ROT and RAN_TRA) with the response pattern of the respective anticipated perturbation (i.e., ROT and TRA).

Statistics

The EMG responses to PNS (M-wave, H-reflex) and TMS (MEP, SICI) prior to perturbation onset were analyzed using a one-way repeated measures ANOVAs with the

factor CONDITION (ROT vs. TRA vs. RAN vs. STA). To ensure that changes in these evoked responses were not attributed to differences in initial posture (i.e., background muscle activity), the bEMG for these same trials prior to stimulation were also analyzed using one-way repeated measures ANOVAs with the factor CONDITION (ROT vs. TRA vs. RAN vs. STA). In case of significant effects between conditions, Pearson's correlation coefficients (r) were computed for the difference in stimulation response between conditions and the difference in bEMG activity (i.e., ΔbEMG) between conditions to check the influence of the bEMG. In case of significant H-reflex values between conditions, correlations were calculated between the changes in the H-reflex (i.e., ΔH-reflex) and changes in the M-wave (i.e., ΔM-wave) of two conditions to confirm that adaptations in the H-reflex were not caused by altered stimulation parameters (as reflected by the M-wave amplitude).

The effect on anticipation on the behavioral responses, specifically the SLR, MLR and LLR from the SOL, the TA EMG response, as well as the joint kinematics, were compared using Student's t-tests between the anticipated and the non-anticipated conditions (e.g., ROT vs. RAN_ROT and TRA vs. RAN_TRA, respectively). Only the CON trials were considered as these trials did not involve any stimulation-evoked muscle response. One-way repeated measures ANOVAs with the factor PERTURBATION (ROT vs. TRA vs. RAN) were conducted to examine whether the ANG and bEMG (i.e., initial standing posture) were different between the three perturbation conditions (ROT vs. TRA vs. RAN).

Effect sizes for all ANOVAs are presented as partial eta square values (η_p^2; small effect: 0.02; medium effect: 0.13; large effect: 0.26). In case of significant F-values ($p \leq 0.05$), post-hoc analysis with Bonferroni-corrected Student's t-tests was applied to determine significant differences between factor levels. Effect sizes for Student's t-test and correlations are reported as Pearson's correlation coefficient values (r; small effect = 0.10; medium effect = 0.30; large effect = 0.50). All statistical analyses were executed in SPSS Statistics 23 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA). The alpha level was set at ≤0.05 and data are presented as group mean values ± standard deviation.

RESULTS

Neuromuscular activity during preparatory setting

Both the SOL ($F_{3,42}$ = 3.536; p = 0.023; η_p^2 = 0.20; see Fig. 2) and TA bEMG ($F_{3,42}$ = 4.241; p = 0.010; η_p^2 = 0.23) revealed a significant effect for CONDITION. Although it appeared that this difference was a result of a decreased bEMG during the STA trials, indicating that muscular activity was reduced when no perturbations were expected, Bonferroni-corrected post-hoc t-tests revealed no differences in SOL or TA bEMG between any two conditions (Fig. 2).

When PNS was applied 70 ms prior to a perturbation, the SOL H-reflex was significantly different between conditions ($F_{3,42}$ = 3.518; p = 0.023; η_p^2 = 0.20; Fig. 3). Post-hoc tests revealed larger amplitudes when the H-reflex was elicited before translations compared to rotations (ROT vs. TRA: p = 0.048; r = 0.64). No significant correlations were found for ΔbEMG and ΔH-reflex between ROT and TRA (p = 0.647; r = 0.12), indicating that the H-reflex was not altered due to changes in the standing background muscle activity. Statistical analysis of the M-wave amplitudes revealed no differences between conditions ($F_{3,42}$ = 2.336; p = 0.087; η_p^2 = 0.14). Nevertheless, a correlation analysis was assessed to check whether the size of the M-wave influenced H-reflex amplitudes. A significant negative correlation was detected for the ΔM-wave and ΔH-reflex between ROT and TRA (p = 0.026; r = −0.54), demonstrating that increased M-wave amplitudes in ROT compared to TRA were associated with decreased H-reflex amplitudes in most participants. Therefore, changes in the H-reflex amplitude were unlikely to be a result of alterations in stimulation parameters.

No effects were found for the SOL peak-to-peak control MEP values ($F_{3,42}$ = 0.435; p = 0.729; η_p^2 = 0.03) when stimulating 70 ms prior to a perturbation. In contrast, control MEP values in the TA were significantly different between conditions ($F_{3,42}$ = 9.732; p < 0.001; η_p^2 = 0.41;). Post-hoc analysis revealed significantly smaller TA MEPs for STA compared to RAN (p < 0.001; r = 0.89), ROT (p < 0.001; r = 0.89), and TRA (p = 0.050; r = 0.63). Correlations for adaptations of bEMG and MEP in TA were apparent between RAN and STA (r = 0.62, p = 0.013) and TRA and STA

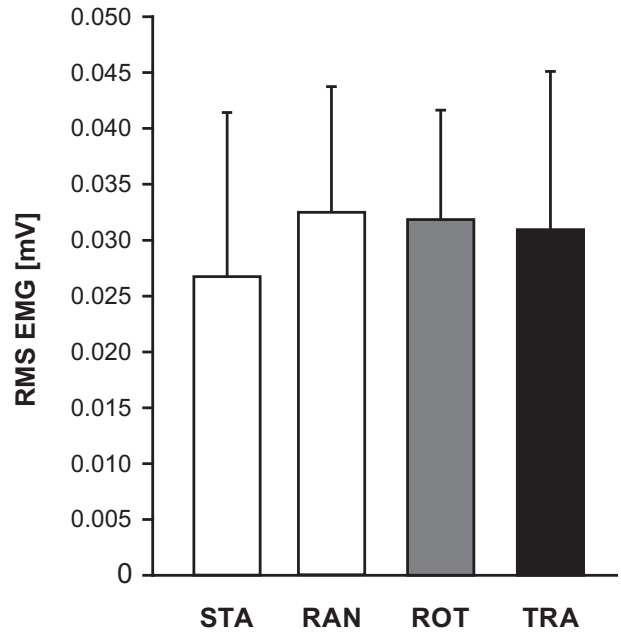

Fig. 2. Mean group soleus (SOL) background EMG (bEMG) for each experimental condition. bEMG was determined during the anticipatory preparation phase (i.e., 80–130 ms before perturbation onset). STA: Static upright stance without perturbation. RAN: Random involving both rotation and translation. ROT: Blocked rotation. TRA: Blocked translation. No differences between conditions were found when using Bonferroni-corrected post-hoc t-tests.

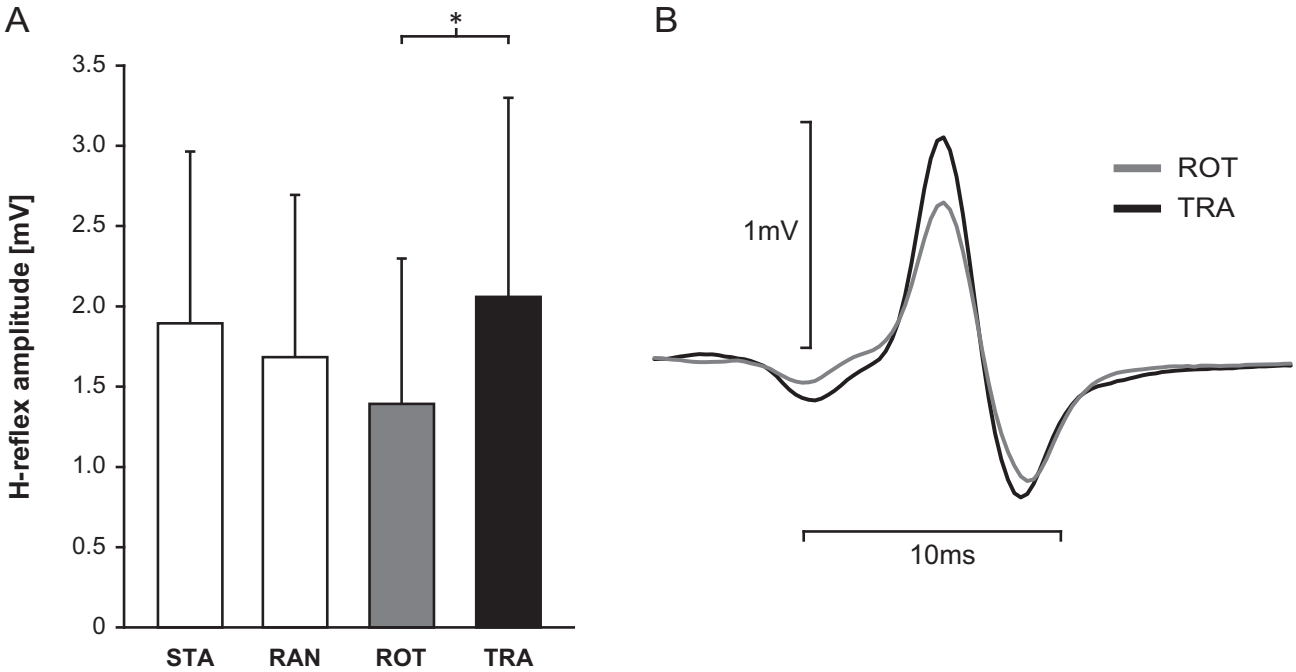

Fig. 3. SOL H-reflexes elicited by peripheral nerve stimulation (PNS) 70 ms before perturbation. (A) Group mean peak-to-peak EMG amplitudes for each experimental condition. (B) Mean H-reflex size before blocked rotational (ROT; gray line) and blocked translational (TRA; black line) perturbations from one representative participant. STA: Static upright stance without perturbation. RAN: Random involving rotation and translation. ROT: Blocked rotation. TRA: Blocked translation. $^{*}p < 0.05$.

($r = 0.64$, $p = 0.010$), but not between ROT and STA ($r = 0.45$, $p = 0.096$).

The statistical analysis for SICI indicated main effects for SOL ($F_{3,42} = 13.725$; $p < 0.001$; $\eta_p^2 = 0.50$; Fig. 4) and TA ($F_{3,42} = 10.253$; $p < 0.001$; $\eta_p^2 = 0.42$). Compared to STA, SICI in the SOL was reduced for all conditions involving a perturbation: RAN ($p = 0.001$; $r = 0.80$), ROT ($p = 0.004$; $r = 0.76$), and TRA ($p < 0.001$; $r = 0.84$). Similarly, SICI in the TA was greater in STA than in the three conditions with a perturbation (RAN vs. STA: $p = 0.005$; $r = 0.69$, ROT vs. STA: $p = 0.033$; $r = 0.67$, TRA vs. STA: $p = 0.008$; $r = 0.66$). No significant Pearson's correlation coefficients were detected for adaptations in bEMG and SICI between STA and the three perturbed conditions for SOL and TA.

Neuromuscular activity after the onset of perturbation

All comparisons of SOL bEMG ($F_{2,28} = 0.191$; $p = 0.827$; $\eta_p^2 = 0.01$), TA bEMG ($F_{2,28} = 2.083$; $p = 0.143$; $\eta_p^2 = 0.13$), and ANG ($F_{2,28} = 0.574$; $p = 0.570$; $\eta_p^2 = 0.04$) in perturbed conditions only (i.e., RAN, ROT, and TRA) were not significant, indicating that the initial muscular activity and the ankle joint angles were not different across conditions. Once the perturbation was initiated, the group mean SOL EMG activity following perturbation onset is shown in Fig. 5. When the effect of anticipation on EMG RMS amplitude was examined for each perturbation type, no differences were detected for the SLR and MLR for either the rotational or translational perturbations. However, the SOL LLR was significantly decreased in ROT and increased in TRA compared to the non-anticipated conditions (i.e., RAN_ROT and RAN_TRA; see Fig. 5 and Table 1). For the TA, EMG activity was significantly reduced in the anticipated ROT

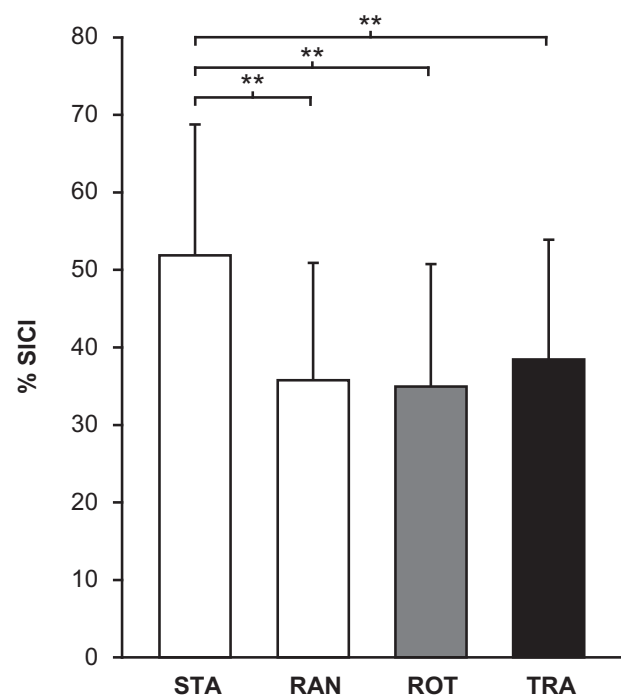

Fig. 4. Group mean percentage differences between mean peak-to-peak EMG amplitude of single and double pulse TMS 65 ms before perturbation of SOL for each experimental condition. STA: Static upright stance without perturbation. RAN: Random involving rotation and translation. ROT: Blocked rotation. TRA: Blocked translation. SICI: Short-interval intracortical inhibition. $^{**}p < 0.01$.

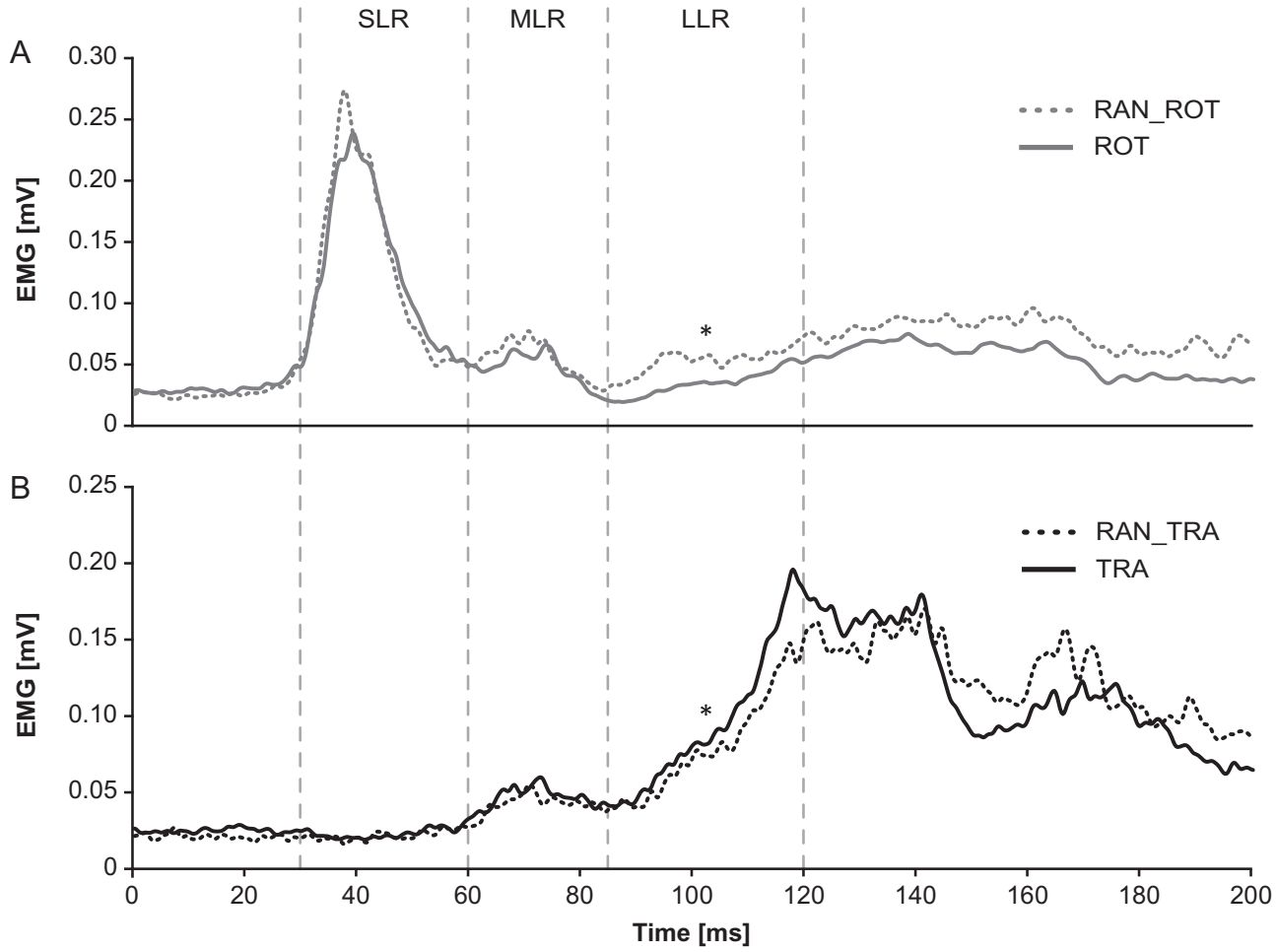

Fig. 5. Group mean EMG signal of m. soleus (SOL) after CON perturbations. Zero on the *x*-axis represents the onset of perturbation and dotted light gray vertical lines indicate the analyzed time windows. (A) EMG activity of SOL after RAN_ROT (dotted gray line) and ROT (gray line). (B) EMG activity of SOL after RAN_TRA (dotted black line) and TRA (black line). SLR: Short-latency response. MLR: Medium-latency response. LLR: Long-latency response. $^{*}p < 0.05$.

Table 1. Student's *t*-tests (*p*) and effect sizes (*r*) of root mean square (RMS) EMG values after anticipated (ROT, TRA) and non-anticipated (RAN_ROT, RAN_TRA) rotational and translational perturbations. SLR: Short-latency response of m. soleus (SOL). MLR: Medium-latency response of SOL. LLR: Long-latency response of SOL. TAR: Response of m. tibialis anterior between 120 and 200 ms after perturbation. mANG: Maximal ankle joint angle after perturbation. tANG: Time point of mANG. Results are indicated as group mean values ± standard deviation

	Rotation				Translation			
	Anticipated ROT	Non-anticipated RAN_ROT	*p*	*r*	Anticipated TRA	Non-anticipated RAN_TRA	*p*	*r*
SLR [mV]	0.183 ± 0.074	0.189 ± 0.087	0.574	0.15	0.029 ± 0.014	0.034 ± 0.009	0.170	0.36
MLR [mV]	0.059 ± 0.037	0.069 ± 0.039	0.138	0.39	0.059 ± 0.027	0.060 ± 0.014	0.840	0.05
LLR [mV]	0.043 ± 0.015	0.067 ± 0.033	0.002	0.72	0.136 ± 0.049	0.116 ± 0.038	0.046	0.51
TAR [mV]	0.412 ± 0.165	0.524 ± 0.160	0.004	0.68	0.054 ± 0.046	0.073 ± 0.054	0.127	0.40
mANG [°]	9.57 ± 2.71	11.85 ± 4.16	0.008	0.65	4.76 ± 0.89	4.89 ± 0.92	0.648	0.12
tANG [ms]	426 ± 228	490 ± 178	0.177	0.36	208 ± 18	234 ± 72	0.166	0.37

condition compared to the non-anticipated RAN_ROT condition during the 120- to 200-ms time window (see Fig. 6 and Table 1). Furthermore, mANG was significantly smaller when participants were able to anticipate compared to not anticipate an upcoming rotational perturbation (i.e., ROT compared to RAN_ROT; see Table 1).

DISCUSSION

The current study investigated how individuals altered their preparatory setting at the spinal and cortical levels prior to an anticipated perturbation. Similar to previous studies (Nashner, 1976; Horak et al., 1989), we demonstrated that the muscular responses in the agonist were

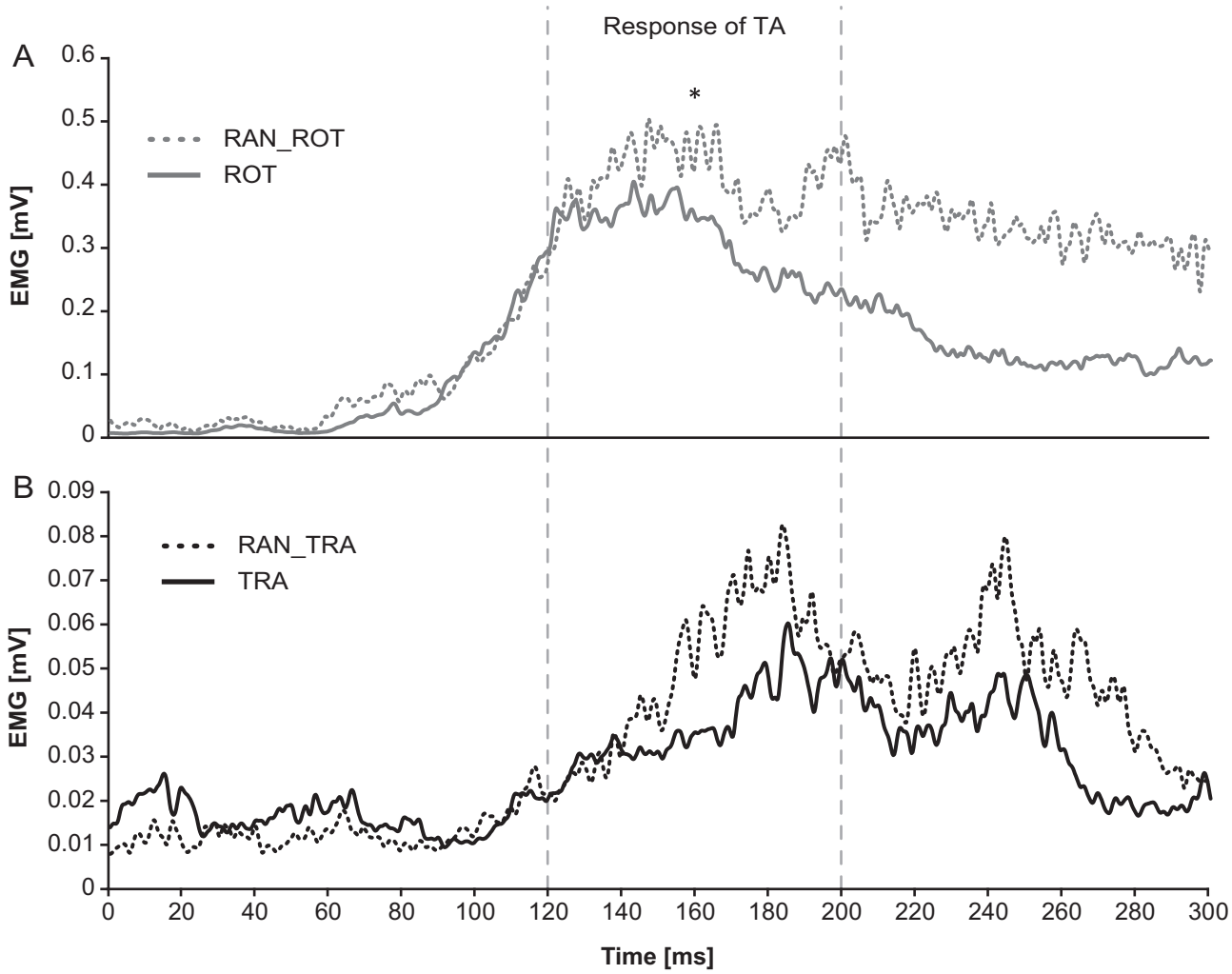

Fig. 6. Group mean EMG signal of m. tibialis anterior (TA) after the CON perturbations. Zero on the *x*-axis represents the onset of perturbation and the dotted light gray vertical lines indicate the analyzed time window. (A) EMG activity of TA after RAN_ROT (dotted gray line) and ROT (gray line). (B) EMG activity of TA after RAN_TRA (dotted black line) and TRA (black line). $^{*}p < 0.05$.

modulated in a functional and perturbation-specific way when the type of perturbation could be anticipated. However, unlike previous studies, this study is the first to use PNS and TMS to investigate the neural presetting prior to perturbation onset.

Preparatory setting

At the spinal level, significantly smaller H-reflexes during preparatory setting in ROT compared to TRA were detected. Since stretch reflexes of calf muscles are detrimental to counteract rotational perturbations but beneficial in translational movements (see also Nashner, 1976), it appears that spinal excitability was modulated in a perturbation-specific manner. Decreased H-reflex amplitudes prior to ROT compared to TRA would reflect altered Ia-afferent transmission in those two conditions. A reduced Ia-transmission can be assumed to be beneficial for counteracting ROT whereas a facilitated Ia-transmission should help to compensate for a TRA perturbation. As both the sizes of the M-wave and the bEMG were not positively correlated with the H-reflex amplitudes between ROT and TRA, it might be assumed that the modulation of the monosynaptic reflex was driven by presynaptic inhibition (Hultborn et al., 1987), which in turn was probably controlled by supraspinal centers (Katz et al., 1988). Thus, the current study is the first to describe perturbation-specific preparatory adjustments of the H-reflex. It has to be noted that both ROT and TRA corresponded to an anticipated test design. Comparing anticipated (ROT and TRA) and non-anticipated (RAN) situations, however, did not reveal statistically significant differences between conditions. Nevertheless, the general trend of preparatory H-reflex amplitude modulation was that the non-anticipated (i.e., RAN) and non-perturbed (i.e., STA) conditions were in between the sizes of the ROT and TRA H-reflexes (see Fig. 3A). This H-reflex presetting in a "medium state" in non-anticipated perturbations and during stance seems functionally adequate as it may allow immediate up- or down-regulation of the reflex response.

It was previously demonstrated that MEPs (Duque et al., 2017) and SICI (Reynolds and Ashby, 1999; Hummel et al., 2009) were reduced shortly before the onset of voluntary movements. In contrast, MEP sizes

that were recorded shortly before the onset of non-voluntary movements (e.g., responses to perturbations) were not modulated (Petersen et al., 2009). Our results – similar SOL MEP-sizes across all three perturbation conditions and during upright stance – are in line with this finding, which suggest that corticospinal excitability was not altered during the preparatory setting.

Altered preparatory setting between conditions was however observed at the cortical level, where SOL and TA SICI were significantly higher during quiet standing compared to the three perturbation conditions. This finding is in line with previous research showing that SICI is reduced when postural demands are increased (Papegaaij et al., 2016a). Furthermore, intracortical inhibition and corticospinal excitability were demonstrated to be reciprocally modulated (Papegaaij et al., 2016a,b), indicating that inhibitory mechanisms are reduced whenever cortical contribution is enhanced. It is therefore assumed that the downregulation of SICI is important to prepare an adequate (cortical) response to the upcoming perturbation. In this context, it could be argued that changes in SOL bEMG influenced the modulation of SICI as previous studies have shown that SICI is reduced when muscles are contracted voluntarily (Ridding et al., 1995; Ortu et al., 2008). However, as there were no significant correlations for bEMG and SICI between STA and the perturbed conditions (i.e., RAN, ROT, and TRA), reduced SICI while standing was unlikely to be caused by lower bEMG levels.

Greater EEG amplitudes before temporally predictable non-anticipated (i.e., randomized design of constrained and non-constrained forward falls) compared to anticipated perturbations (i.e., blocked design) have previously been reported (Mochizuki et al., 2010). Based on this and the fact that SICI is known to be decreased in more challenging postural tasks (Papegaaij et al., 2016a), we initially hypothesized that SICI would be reduced in the RAN compared to the anticipated conditions. However, our results did not support this hypothesis. One reason for this might originate from our study design. Whereas Mochizuki and colleagues (2010) applied one perturbation type with two amplitudes in the non-anticipated conditions, the present study used two different types of perturbations (ROT and TRA) that required contrary compensatory muscular responses (inhibition and facilitation, respectively). It might therefore be assumed that the preparatory setting of SICI in the RAN condition was adjusted to a "medium state" so that both rotations and translations could be accomplished. Alternatively, the timing of our magnetic stimulation might have been too early so that changes in SICI were not yet pronounced enough 70 ms before the perturbation. Although the present results did not show an adaptation of SICI in response to the type of perturbation or predictability, they are nevertheless adding more knowledge within this field by indicating that the preparatory setting in higher brain centers involves altered levels of intracortical inhibition as soon as a perturbation is expected.

Reactions in response to the perturbation

The behavioral consequences of preparatory setting in response to predictability were examined by comparing postural and muscular responses. No significant differences in SLR and MLR in response to rotational and translational perturbations were observed between anticipated and non-anticipated perturbations. It is therefore assumed that the preparatory setting of spinal responses such as the SLR and MLR are not sensitive to the discrimination of anticipated versus non-anticipated perturbations, i.e., the predictability. Perturbation-specific comparisons of the SLR between ROT and TRA were not conducted as the response pattern of the two types of perturbation was quiet different (see Fig. 5). In contrast, H-reflexes evoked before perturbation onset were shown to be modulated in a perturbation-dependent manner during the anticipated conditions (i.e., ROT vs. TRA). With regard to cortical responses, the ability to anticipate the type of perturbation resulted in a downregulation of the LLR in ROT and an upregulation in TRA compared to the corresponding non-anticipated perturbations (RAN_ROT and RAN_TRA, respectively; see Fig. 5). This result highlights the quality of the LLR to properly adapt to the requirements to counteract the respective perturbation when the perturbation can be anticipated. Adaptations depending on preparatory setting have previously been reported in the gastrocnemius muscle in response to backward translations (Horak et al., 1989). The authors concluded that "...effects are most prominent on the earliest component of a triggered response...(Horak et al., 1989)". However, based on the current results, this statement should be reconsidered. First, the earliest components of the triggered responses in ROT of the present study – the SLR and MLR – were rarely affected. Thus, these observations suggest that the preparatory setting in response to predictability is primarily modulating cortically mediated reflex responses (i.e., the LLR) but not necessarily the spinally generated bursts of muscle activation. In addition, previous studies examining the impact of preparatory setting on postural responses referred to muscular activity onset latencies of around 100 ms (Horak et al., 1989) and 120 ms (Nashner, 1976) after perturbation. Since it was shown that transcortical pathways are involved in muscular responses from 86 ms onwards after the postural disturbance (Taube et al., 2006), it is assumed that the first muscular activity in these previous studies are LLRs mediated by a transcortical loop. The non-occurrence of short- and medium-latency responses in those studies can plausibly be explained by the usage of much slower perturbations than in the current study. This further strengthens our assumption that the preparatory setting primarily affects cortically mediated responses. In line with this, it was previously reported that cortical activity assessed with EEG was different between predictable and unpredictable perturbations at around 100 ms after onset of perturbation (Adkin et al., 2006).

TA EMG activity started to increase at around 100 ms after perturbation onset while the LLR in SOL was simultaneously active. Subsequent muscular activity in TA (i.e., response of TA; 120–200 ms after perturbation onset) was decreased in anticipated compared to non-anticipated situations in both perturbation types (see

Fig. 6). However, the reduction was only statistically significant for the rotational perturbations. Prior research provided evidence that the antagonist is primarily modulated by postural sway and not by the induced muscle spindle stretching of the agonist after perturbations (Nardone et al., 1990). Increased muscular activity in TA after non-anticipated rotations (i.e., RAN_ROT) is therefore suggested to be a consequence of greater postural sway compared to an anticipated situation (i.e., ROT). This view is reinforced by the analysis of the ankle angle kinematics. Maximal ankle angle movements (mANG) were significantly larger in RAN_ROT compared to ROT (11.85 ± 4.16° vs. 9.57 ± 2.71°; $p = 0.008$). The reason for this larger ankle angle could be seen in kinematic recordings showing that some subjects had to lift up the frontal part of their feet to stabilize posture in the RAN_ROT condition. In contrast, when the rotation could be anticipated, participants did not have to lift their feet up off the platform.

The present study highlighted that intracortical inhibition is reduced during the preparatory setting as soon as participants expect any kind of perturbation. Furthermore, H-reflexes elicited shortly before perturbation onset were increased in anticipated translations and decreased in anticipated rotations, indicating that the preparatory setting affects spinal Ia-afferent transmission in a perturbation-specific manner. However, the preparatory setting at the spinal level did not depend on predictability (i.e., between anticipated and non-anticipated perturbations). Adapted responses in the anticipated conditions were limited to the cortically mediated LLR. Therefore, the current results suggest that the preparatory setting at the spinal level takes into account the type of perturbation, whereas the preparatory setting at the cortical level is influenced by whether a perturbation can be anticipated or not.

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