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Differences in motor cortical control of the Soleus and Tibialis

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Summary statement

This paper investigated differences in the cortical control of the soleus and tibialis anterior muscles highlighting profound differences during rest as well as during activity

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Abstract

The tibialis anterior (TA) and the soleus (SOL) are both ankle joint muscles with functionally very different tasks. Thus, differences in motor cortical control between the TA and the SOL have been debated. This study compared the activity of the primary motor cortex during dynamic plantar- and dorsiflexions and compared this with measures obtained during rest. Single- and paired-pulse transcranial magnetic stimulations known as short-interval intracortical inhibition (SICI) were applied to the cortical representation of either the soleus or the tibialis muscle. The results show that the range of SICI from rest to activity is significantly greater in the TA compared with the SOL. Furthermore, when the TA acts as the agonist muscle during dorsiflexions of the ankle, SICI is almost absent (2.9%). When acting as the antagonist during plantarflexions, intracortical inhibition is significantly increased (28.7%). This task-specific modulation is far less pronounced in the SOL, which displayed higher levels of SICI when acting as agonist (10.9%) during plantarflexion, but there was no significant inhibition (6.5%) as antagonist during dorsiflexion. Furthermore, the cortical silent period (CSP) during plantarflexions was significantly longer in the SOL compared with the TA during dorsiflexions, accompanied by a greater corticospinal excitability in the TA. Thus, cortical control considerably differs between the SOL and the TA in a way that inhibitory cortical control (SICI and CSP) of the TA is task-specifically adapted in a broader range of movements, whereas inhibition in the SOL muscle is less specific and more limited in its magnitude of modulation.

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Introduction

From a functional perspective, both, the soleus (SOL) and the tibialis anterior (TA) muscles are crucial for ankle joint motions, but they differ in their function: while the SOL has the potential to produce high forces (e.g. during walking), forces generated by the TA seem to be considerably lower (Lieber and Friden, 2000). During walking, the precision when lifting the foot over ground during the swing phase was previously assumed to require very fine motor (cortical) control of the TA (Petersen et al. 2003), whereas the control of the SOL muscle may rather be reactive and therefore less defined (Capaday et al., 1999). Thus, it seems to be likely that the specific functional roles of the two muscles require distinctive motor cortical control, but it is still unclear whether there are differences in motor cortical control between the dorsiflexor TA and the plantarflexor SOL. The first studies comparing the tibialis anterior with the soleus by applying transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) proposed that there exist considerable differences in the responses following cortical stimulation. It was, for example, shown that in the SOL, TMS resulted in less clear responses than when the TA was stimulated. It was argued that this is because the SOL motoneurons receive weaker or less meaningful corticomotoneuronal input (Brouwer and Qiao, 1995; Brouwer et al., 1992) even though it has been shown that the motoneurons of the plantarflexor (SOL) and the dorsiflexors TA receive monosynaptic (direct) corticospinal inputs (Matthews, 1991; Valls-Sole et al., 1994). Nielsen and Petersen (1995) reported poorly defined peaks in post stimulation time histograms (PSTHs) in the SOL, while the TA motor units showed clear responses. Later evidence, however, did not support the notion that there is a lack of systematic corticomotoneuronal connections on SOL motoneurons compared with the TA. First, de Noordhout et al. (1999) showed a similar projection of corticomotoneuronal synapses onto the two pools by determining the rise time of monosynaptic excitatory postsynaptic potentials in the TA (1.13ms) and SOL (1.14ms). Thereafter, in Bawa et al. (2002), TMS was applied to the leg area of the left motor cortex while responses were measured using surface electromyography (EMG) together with single motor unit recordings. While the authors demonstrated differences in the onset of the motor evoked potentials (MEP) during rest, they failed to identify significant differences during isometric contractions or when the TA tendon was vibrated. Thus, Bawa et al. (2002) concluded that there is no systematic difference in the presence of corticomotoneuronal connections even though the authors admit that the strength of these connections might be higher in the TA. However, apart from the corticomotoneural connections, the cortical representations of TA and SOL may also differ. For example, a study using functional magnetic resonance imaging (fMRI) showed that dorsiflexions of the ankle

Journal of Experimental Biology • Accepted manuscript

resulted in much higher levels of activity in motor cortical areas compared with plantarflexions (Trinastic et al., 2010) . Furthermore, Papegaaij et al. (2016) demonstrated that during upright stance, short-interval intracortical inhibition (SICI) was smaller in the SOL (≈ 50% inhibition) than in the TA (≈ 70% inhibition). However, as the study of Papegaaij et al. (2016) specifically focused on the SOL but not the TA, it is difficult to make direct assumptions about differences between the two muscles with respect to cortical inhibitory control.

Accordingly, the aim of the present study was to systematically investigate differences in motor cortical control of SOL and TA. For this purpose, transcranial magnetic stimulation (TMS) was applied to the cortical representations of the SOL and the TA during activity (plantarflexions, dorsiflexions) and at rest. A paired pulse TMS protocol, where the first stimulus is subthreshold while the second one is suprathreshold, was chosen to assess short-interval intracortical inhibition (SICI). There is strong evidence from studies with patients in which descending corticospinal activity was recorded directly from the spinal cord that SICI is a cortical phenomenon (Di Lazzaro and Rothwell, 2014; Di Lazzaro et al., 1998; Ni et al., 2011; Weise et al., 2013). These studies showed a suppression of later I-waves (synaptically evoked corticospinal volleys) by the conditioning pulse when the primary motor cortex is stimulated by subthreshold intensities (Di Lazzaro et al., 1998; Nakamura et al., 1997), while there were no changes occurring for D-waves (direct corticospinal volleys). From a functional perspective, it is assumed that high levels of SICI prior to the start of a movement are important to supress corticospinal excitability and prevent unwanted or premature movements (Levin et al., 2014). In contrast, the release of SICI before and during the actual movement allows for a rapid facilitation of corticospinal excitability in the target muscle(s), probably leading to a more synchronous activation (Beck and Hallett, 2011). We tested SICI during dynamic contractions where low levels of inhibition are important in the agonist to provide high levels of cortical drive, while inhibition in the antagonist should be high to prevent counterproductive co-activation of muscles. Importantly, stimulation intensities were muscle- and task-specifically adjusted in the present study. Furthermore, TMS stimulation was triggered on the background EMG, guaranteeing comparable background EMG levels so that direct comparisons between muscles and conditions could be made. Brain stimulation was not only applied during activity but also at rest in order to assess a baseline level of inhibition. This made it possible to determine the range of inhibitory modulation from the resting condition (sitting) to the active condition in the same posture (plantar- and dorsiflexions). Furthermore, the range of inhibitory modulation was measured by comparing the amount of inhibition when the muscle acted as agonist and as

Journal of Experimental Biology • Accepted manuscript

antagonist. Furthermore, additional measures such as cortical silent period and corticospinal excitability were collected in order to evaluate a greater spectrum of differences in cortical control between the TA and the SOL. Based on the abovementioned results obtained with double-pulse TMS (e.g. Papegaaij et al., 2016), it was hypothesized that the TA has a greater potential to modulate inhibition in a task-specific manner than the SOL.

Materials and Methods

Study participants

A total of 27 subjects (23.2 ± 2.9 years, 13 female) volunteered for this study. Statistical power was assured by *a priori* power analyses using the G*power software (Faul et al., 2007). Before the actual experiment, subjects read the information sheet about the content of the study and then gave written informed consent. The study was approved by the ethics committee of the University of Freiburg (418/16) and was in accordance with the latest version of the Declaration of Helsinki.

Electromyography (EMG)

After shaving and cleaning the skin with disinfectant, surface EMG was taken from the m. soleus (SOL) and the m. tibialis anterior. Surface electrodes (Blue sensor P, AmbuH, Bad Nauheim, Germany) were attached to the muscles according to SENIAM guidelines with an interlectrode distance of 2cm. The reference electrode was placed on the tibial plateau. The EMG recordings were amplified (x 500), bandpass filtered (10–1000 Hz), and sampled at 4kHz.

Transcranial magnetic stimulation (TMS)

Transcranial magnetic stimuli were applied over the left hemisphere motor cortex using a 95mm focal butterfly-shaped coil (D-B80) and a MagPro X100 with MagOption magnetic stimulator (both MagVenture A/S, Farum, Denmark). The coil was attached to a custom-made helmet (Petzl, Garmisch-Partenkirchen, Germany) that could be adjusted to different head sizes. The coil could be moved relative to the head via a pin-jointed system made of lightweight but rigid plastic. The helmet and the coil were additionally secured by straps to the chin and the back of the head (Taube et al., 2008). To minimize the forces acting on the helmet via the cable of the coil, the cable was separately fixed via a pulley system attached to the ceiling above the subjects. For every subject, the initial stimulation point was set approximately 0.5cm anterior to the vertex and over the midline. Then, the final position of the coil was established by moving

Journal of Experimental Biology • Accepted manuscript

the coil anterior and left from the vertex while constantly monitoring the size of the motor evoked potential (posterior-anterior current flow in the motor cortex, with the handle pointing backwards).

Intracortical inhibition

For the present study, intracortical inhibition was tested using a paired-pulse TMS protocol in which the suprathreshold single TMS pulse at 1.2MT was preceded by the subthreshold (0.8MT) TMS pulse by 2.5ms (Kuhn et al., 2017; Papegaaij et al., 2016). While the first pulse acts as a conditioning pulse being delivered at intensities below the threshold to evoke an MEP (subthreshold TMS), the second pulse actually evokes a clearly visible MEP (supratheshold TMS). The first pulse activates intracortical inhibitory interneurons that then reduce the MEP amplitude of the second impulse, and this phenomenon is known as short-interval intracortical inhibition (SICI). The peak-to-peak amplitude of the conditioned MEP is then compared to the unconditioned MEP. In the present study, SICI was tested during rest as well as during activity. Due to the changes in the level of muscle activation from rest to activity, the stimulation intensity was adjusted for each condition and muscle (please see below). This procedure ensured that there was no visible MEP following the subthreshold TMS during the rest (control) nor during the active conditions. In contrast, the suprathreshold TMS resulted in a clear visible MEP in the SOL and the TA, respectively.

Resting motor threshold (RMT)

The RMT was defined as the lowest possible stimulation intensity to evoke MEPs greater than 50μV in three out of five consecutive trials obtained during rest (Rossini et al., 1994). The optimal position of the coil was marked on the subject's head using a felt pen and monitored throughout the entire experiment. The RMT was individually established for the SOL and the TA.

Active motor threshold (AMT)

Active motor threshold (AMT) was defined as the stimulation intensity to evoke MEPs greater than 100μV in three out of five consecutive trials (Papegaaij et al., 2014) during the dynamic plantar- (SOL) and dorsiflexions (TA). The AMT was individually established for the SOL and the TA while subjects performed the plantar- or dorsiflexions. To establish the AMT, a target representing 70% of the maximal force was represented on a computer screen in front of the subjects as a red target line that had to be matched by a black line representing the exerted force

Journal of Experimental Biology • Accepted manuscript

of the subjects. Subjects were instructed to contract as fast as possible (Maffiuletti et al., 2016) and match the target line as closely as possible. The TMS stimulation was triggered on the rising EMG corresponding to 50% of the maximal rectified EMG obtained during the submaximal contractions. This level for triggering the EMG has been successfully used previously to study the influence of SICI during cycling (Sidhu et al., 2013), and we therefore adapted this paradigm. This trigger-level was kept constant throughout the entire experiment to ensure the same level of muscle activation at the time of stimulation.

SICI during plantar- and dorsiflexions

After establishing the AMT for the SOL and the TA, subjects performed 30 submaximal isometric ballistic contractions (please see below) during which 15 paired-pulse stimulations (SICI) and 15 single-pulse MEPs were applied in a randomized order with the subthreshold TMS pulse at 0.8 AMT followed by the suprathreshold stimulus at 1.2 AMT. Importantly, stimulation intensity was individually adjusted for each muscle and condition (0.8 & 1.2 AMT) depending on whether the subjects performed plantarflexions (SOL) or dorsiflexions (TA). Although the primary aim was to elicit MEPs and SICI in the agonistic muscles ($_{ago}$), MEPs were simultaneously recorded in the antagonist muscles ($_{ant}$), as well. Thus, data from the SOL_{ant} and the TA_{ant} is also reported, but they need to be interpreted with caution as TMS intensity was not adjusted for the antagonist but for the agonistic muscles.

SICI in control conditions (rest)

To test the amount of intracortical inhibition during rest, 15 single- and 15 paired-pulse TMS stimulations around RMT were applied in the same posture as in the active condition (sitting) in the SOL as well as in the TA. We included the rest conditions because it allowed us to measure the baseline level of inhibition so that it was possible to calculate the range of how SICI is modulated between rest and activity for each condition.

Plantar- and dorsiflexions

For the isometric plantar- and dorsiflexions, subjects were seated in a comfortable but rigid chair with the hip at 90 degrees and the knee at 180 degrees (fully extended). The foot was at an ankle angle of 100 degrees and attached to the footplate of a custom-made device measuring ankle torque (Ritzmann et al., 2013). The foot was strapped to the footplate to exclude movements of the ankle. The hip and the trunk of the subjects were strapped to the backrest of the chair to avoid trunk movements. The seating area of the chair was large enough to provide

Journal of Experimental Biology • Accepted manuscript

some support of the thigh, while the shank was unsupported. For familiarization, subjects were allowed to perform a set of 5-7 submaximal contractions. Subsequently, subjects performed 5 contractions and were instructed to contract as fast and as hard as possible for approximately 1 second and were allowed to rest for 30 seconds between the contractions (Maffiuletti et al., 2016). The actual force and the target level were visually displayed on a computer screen placed 2m in front of the subjects, and the subjects were instructed to initiate the contractions according to the beat of a metronome indicating the start of a new contraction every 20s. In addition, subjects were instructed to contract as fast as possible. Data was sampled at 4kHz.

Control experiment for the antagonistic muscles

In order to test whether the differences in SICI in the antagonist muscles were biased, a control experiment was conducted in six subjects (27.3 ± 4.2 years, all male). During this control experiment, subjects performed dynamic plantar- or dorsiflexions, while SICI was measured in the agonist as well as the antagonist muscle. This time, however, the coil position and stimulation intensity were adjusted specifically to task and muscle. This means that when the SOL acted as antagonist, TMS was applied during the dynamic dorsiflexion, but the coil position and stimulation intensity were not adjusted to evoke MEPs in the TA but in the SOL. When the TA acted as antagonist, TMS was applied during dynamic plantarflexions, and the coil position and stimulation intensity were muscle specifically adapted to the TA. When the SOL and TA acted as agonists, TMS measurements were carried out in the same way as in the main experiment (described under *SICI during plantar- and dorsiflexions)*, i.e. the coil position and stimulation intensity were adjusted to the respective agonist. RMT and AMT were established in the same way as described above.

Data Analyses and Statistics

Motor cortical inhibition

The size of the motor evoked potential was quantified by peak-to-peak analyses of the conditioned MEP (paired-pulse stimulation) compared to the unconditioned MEP during the active- (plantarflexion, dorsiflexion) and the control conditions at rest (sitting). SICI was expressed as percentage inhibition of the conditioned in relation to the unconditioned MEP using the formula: 100-(conditioned MEP / unconditioned MEP x 100), which has been used previously (Kuhn et al., 2016; Papegaaij et al., 2014).

Journal of Experimental Biology • Accepted manuscript

Corticospinal excitability

Corticospinal excitability (CSE) was quantified by the peak-to-peak amplitude of the MEP resulting from the unconditioned (single-pulse) TMS stimulation measured during the control and active conditions.

Cortical silent period

The cortical silent period (SP) was obtained in the trials with single-pulse stimulation. The duration of the SP was measured from the onset of the MEP, and the end-point coincided with the reoccurrence of EMG activity in individual trials via visual inspection (Kimiskidis et al., 2005).

Background EMG activity (bEMG)

Muscle activation at the time of the TMS stimulation was calculated in a time window of 50ms prior to each stimulation. EMG signals were rectified, and root mean square values were calculated, averaged, and normalized to the maximal EMG activity recorded during the maximal RTD trials.

Statistical comparison

Normal distribution of the data was confirmed using the Kolmogorov-Smirnov test. Then, a repeated measures ANOVA with the factors of muscle (SOL, TA) and condition (rest, active) was calculated when the SOL and the TA acted as agonists. A repeated measures ANOVA with factors condition (plantarflexion, dorsiflexion, rest) and muscle (SO, TA) was calculated to detect differences in SICI when the muscles acted as agonist. When the SOL and the TA were antagonists, a one-way ANOVA was computed to highlight differences in SICI. Differences in the RMT, AMT, unconditioned MEP, SP, and bEMG were calculated with individual one-way ANOVAs. Differences between the RTD during the dorsi- and plantarflexions were compared using a one-way ANOVA. In the case of significant effects, Bonferonni-corrected Student *t*-tests were performed. Pearson's *r* was computed to assess the associations of the RTD and the SICI as well as between the unconditioned MEP amplitude with the SICI. For the control experiment, paired *Student's t-test* was carried out to highlight differences in the muscles when acting as agonist or antagonist.

All data are reported as means ± standard error, and SPSS 24 (Chicago, IL, USA) software was used for all statistical comparisons.

Journal of Experimental Biology • Accepted manuscript

Results

Resting motor threshold (expressed as percentage of the maximum stimulator output)

For the control conditions (rest), the one-way ANOVA showed significant differences in RMT between the muscles ($F_{1,26} = 45.91$, $\eta^2 = 0.\ 03$, $p = 0.41$, $p < 0.001$) with a lower RMT in the TA (57.2 ± 7.2%) than in the SOL (63.5 ± 8.2%).

Active motor threshold (expressed as percentage of the maximum stimulator output)

For the active motor threshold, there was also a significant difference between the SOL and the TA ($F_{1,26} = 9.16$, $\eta^2 = 0.\ 25$, $p = 0.41$, $p = 0.005$) as the AMT during dorsiflexion (45.3% ± 7.2) was significantly lower than the AMT for the SOL during plantarflexion (58.2 ± 7.0%).

Corticospinal excitability

During the active conditions, one-way ANOVA showed that CSE was significantly greater in the TA compared with the SOL ($F_{1,26} = 12.67$, $\eta^2 = 0.32$, $p = 0.001$, Figure 2D) despite similar levels of background activation (see below). Corticospinal excitability was not significantly different between the SOL and the TA ($F_{1,26} = 2.61$, $\eta^2 = 0.09$, $p = 0.12$) during the control conditions (rest).

During the resting condition, the one-way ANOVA showed no significant difference in the size of the unconditioned MEP between the TA *(0.87 ± 0.24mV)* and the SOL (*1.44 ± 0.92mV*, $F_{1,26} = 2.61$, $\eta^2 = 0.09$, $p = 0.12$).

SICI in SOL compared to TA

The repeated measures ANOVA showed no interaction effect of muscle*condition ($F_{1,26} = 0.99$ $\eta^2 = 0.08$, $p = 0.33$). There was, however, a significant difference in SICI between the muscles ($F_{1,26} = 9.85$, $\eta^2 = 0.28$, $p = 0.004$) and the conditions ($F_{1,26} = 68.58$, $\eta^2 = 7.25$, $p = 0.004$). *Post hoc* comparisons showed that compared with the inhibition in the TA during the dorsiflexion (9.1 ± 1.4), SICI was significantly greater in the SOL during plantarflexion (14.9 ± 2.1%, $p = 0.026$, Figure 1A). In contrast, during the control conditions (rest), SICI was significantly greater in the TA (55.2 ± 4.8%) compared with the SOL (44.4 ± 4.8%, $p = 0.028$, Figure 2A).

SICI in the control condition at rest compared to activity

The repeated measures ANOVA showed a significant interaction between the condition (rest vs. active) and the muscles (TA vs. SOL, $F_{3,104} = 15.98$, $\eta^2 = 0.38$, $p < 0.001$). *Post hoc* testing showed that SICI in the SOL was significantly greater during rest compared with plantarflexion

Journal of Experimental Biology • Accepted manuscript

($p < 0.001$). For the TA, SICI was also higher during rest compared with dorsiflexion ($p < 0.001$, Figure 1C). To compare the differences between rest and activity between the two muscles, we also calculated the percentage changes in SICI from the control to the active condition and compared these changes between the SOL and the TA. This analysis revealed a significant difference between the two muscles (one-way ANOVA, $F_{1,26} = 15.00$, $\eta^2 = 0.36$, $p = 0.001$) with the greater modulation from rest to activity in the TA (59.98 ± 4.9%) compared with the SOL (33.23 ± 5.2%; Figure 2B).

Effect of MEP on SICI

To determine whether differences in the size of the unconditioned MEP affected the amount of SICI, correlation analyses of the SICI- and MEP-amplitudes were computed in the active and control conditions at rest (in accordance with Papegaaij et al., 2016). During the active conditions, there was no significant correlation during plantarflexion (SOL, $r = 0.21$, $p = 0.28$) and dorsiflexion (TA, $r = -0.15$, $p = 0.46$). During the control conditions (rest), there was also no significant relationship in the level of SICI and the sizes of the MEPs in the SOL ($r = -0.13$, $p = 0.53$) and the TA during sitting ($r = -0.01$, $p = 0.96$).

SOL and TA acting as antagonists

Even though we did aim to measure the SOL and TA when they acted as agonistic muscles, it was also possible to record MEPs in the two muscles when they acted as antagonists. It needs to be noted, however, that these results need to be interpreted with caution as the TMS intensity and the background EMG was adjusted for the agonistic muscles.

SICI measures in the SOL and TA acting as antagonists

A significant difference between the SOL and the TA was observed when they acted as antagonist muscles (one-way ANOVA, $F_{1,26} = 15.21$, $\eta^2 = 0.36$, $p = 0.01$) as SICI was significantly greater in the TA_{ant} (28.7 ± 4.6%) compared with the SOL_{ant} (6.5 ± 3.6, $p = 0.001$, Figure 3).

Control Experiment

The results from the control experiment follow the same pattern as the results of the main experiment shown under *SICI measures in the SOL and TA acting as antagonists.* When the SOL acted as antagonist, SICI was much lower (14.4 ± 4.7%) compared with when the TA (38.7 ± 6.1%) was acting as antagonist (Figure 4A). When the SOL acted as agonist, SICI was

Journal of Experimental Biology • Accepted manuscript

higher (27.0 ± 6.1%) compared with the TA being the agonist muscle (13.6 ± 4.6%, Figure 4B). Paired Student's t-test shows that there was no significant difference in SOL when acting as agonist or antagonist ($p = 0.17$), while the difference in the TA was significant ($p = 0.005$), supporting the notion of a greater range of how SICI can be modulated in the TA compared with the SOL.

Cortical Silent Period (CSP)

In the active conditions, there was a significant difference in the CSP (mean ± SD; $F_{1,26} = 5.00$, $\eta^2 = 0.16$, $p = 0.03$) with a longer CSP duration in the SOL_{ago} (91.7 ± 2.8ms) during the plantarflexions compared with the TA_{ago} (77.1 ± 2.6ms) during the dorsiflexions (Figure 2C).

Background EMG

The normalized background EMG in the period before the TMS stimulation was significantly different between the conditions (one-way ANOVA plantarflexion versus dorsiflexion; $F_{1,26} = 30.69$, $\eta^2 = 0.60$, $p < 0.001$). There was, however, no significant difference between the normalized EMG values of the SOL (10.3 ± 1.5%) and the TA (11.5 ± 2.0%) when they acted as agonists ($p > 0.90$) as well as when they acted as antagonists ($p > 0.90$, SOL 4.3 ± 0.9, TA 3.4 ± 0.6%). A significantly greater activation was observed in the SOL when acting as agonist vs. acting as antagonist ($p = 0.006$), which was also the case for the TA ($p = 0.008$, Figure 2D).

Rate of torque development

The RTD during the plantarflexions (670 ± 38.91Nm/s) was significantly greater (one-way ANOVA, $F_{1,26} = 203.69$, $\eta^2 = 0.89$, $p = 0.41$, $p < 0.001$) compared with the dorsiflexions (214 ± 15.87Nm/s; Figure 2F).

Discussion

The aim of the present study was to systematically investigate differences in cortical control of the SOL and the TA muscles during dynamic plantar- and dorsiflexion. For this purpose, motor cortical inhibition and corticospinal excitability were tested both during active conditions as well as during control conditions (rest). This allowed us to investigate the muscle-specific range of modulation in cortical inhibition between rest and activity. Overall, the results demonstrate that the task-specific modulation of SICI is more pronounced for the TA. More specifically, in situations in which the TA acts as the agonist, intracortical inhibition is almost absent, while when the TA functions as antagonist, SICI is drastically increased. In contrast, although the

Journal of Experimental Biology • Accepted manuscript

modulation of intracortical inhibition in the SOL is following the same pattern, the task-specific adaptations are less evident. Furthermore, CSP was significantly longer and CSE reduced in the SOL during the plantarflexions compared with the TA during the dorsiflexions, providing new evidence for differences in the motor cortical control of the SOL and TA.

Difference in the active conditions between SOL and TA

For ballistic types of movements such as those investigated in the present study, it was demonstrated that the cortical inhibition is released prior to the generation of the movement (Levin et al., 2014), most likely to allow synchronized high levels of cortical drive. In a study investigating the modulation of SICI in hand muscles, Beck et al. (2008) showed that when subjects pushed against a force transducer, SICI in the agonistic muscle was significantly decreased prior to the movement onset and remained low during the phasic part of the finger movement. This seems to be a general pattern, as it was shown for hand as well as for leg muscles (Chen et al., 1998; Di Lazzaro et al., 2001; Kujirai et al., 1993; Stokic et al., 1997). However, these studies did not compare the modulation of SICI in different muscles. The results of the present study are important, as they demonstrate that the modulation of SICI is changed depending on whether the subjects are required to rapidly activate their SOL or their TA (Figure 1). For the SOL, we observed significant levels of SICI during plantarflexion, whereas SICI was absent in the TA during dorsiflexion. Thus, it seems that the cortical inhibitory control of the TA is better tuned to regulated inhibition depending on the role of the TA. This is further supported by observing how SICI was modulated when the SOL and TA act as antagonists (Figure 3). During plantarflexions, where TA functions as the antagonist, SICI in the TA_{ant} was significantly greater than SICI in the SOL_{ant} during dorsiflexions. Importantly, the results from the control experiment during which the coil position and the stimulation intensity were adjusted to the antagonistic muscles show the same pattern of how SICI is modulated. This supports the notion of a more refined inhibitory control of the TA compared with the SOL. Considering that it is beneficial to have low levels of SICI when the agonistic muscle is expected to produce rapid forces and to have high levels of inhibition to the antagonistic muscle to avoid a counterproductive co-contraction, the task-specific modulation in SICI of the TA was better tuned to the functional needs. Importantly, this seems to be the case in both directions, meaning that SICI was absent when the TA was required to produce rapid forces (dorsiflexion) functioning as the agonistic muscle, but SICI could also be very high and even above the levels of SICI in the SOL when activity in the TA was unwanted, as during plantarflexions. By comparing the activity of the SOL and the TA during walking with the

Journal of Experimental Biology • Accepted manuscript

activity during tonic contractions, Capaday et al. (1999) showed that MEPs in the SOL were lower during the stance phase than during tonic contractions despite matched background EMG levels. Interestingly, the TA displayed larger MEPs during stance compared with the plantarflexion condition, even though the muscle was not very active. It was therefore concluded that during walking, the influence of the motor cortex on the SOL is weaker than for the TA, supporting the notion of a presumed greater cortical control of the dorsiflexors compared with the plantarflexors (Petersen et al., 2003). The present results of higher MEPs in the TA compared with the SOL are congruent to the findings of Petersen et al. (2003), indicating that compared with the SOL, the cortical control of the TA might not only be higher during walking but also during dynamic voluntary initiated plantar and dorsiflexions. However, other factors such as the level of muscle activity and TMS intensity could have affected the results, as both have been shown to influence the size of the MEP and also the amount of SICI (Capaday, 1997; Devanne et al., 1997). To exclude this, we correlated the size of the unconditioned MEP with the size of the conditioned MEP during the control and the active conditions. As there were no significant correlations, it seems unlikely that differences in MEP size might have biased the SICI results.

Resting inhibitory activity vs inhibitory activity during dynamic movements

In order to quantify baseline values of inhibition for each muscle and to assess the relative range of inhibitory modulation from the resting condition to the active condition, we also measured SICI during rest. With respect to the TA, the results from our study differ greatly from values described by Soto et al. (2006), who did not exemplify differences in SICI between the rest and the active condition in this muscle. One reason might be that in contrast to the study of Soto et al. (2006), we established the RMT and AMT for each muscle (SOL and TA) and for each task (rest and active conditions) individually, while Soto and co-workers only concentrated on the SOL. Furthermore, measuring SICI during rest made it possible to calculate the entire range of modulation in SICI between the control and the active condition. The greatest range could be observed in the TA, with 59.98 % difference in SICI, while in the SOL, modulation was only 33.23%. It therefore seems that for the TA, the motor cortex has the potential to regulate SICI from almost no inhibition when acting as the agonist (dorsiflexion) to very high levels of intracortical inhibition when it is acting as antagonist (i.e. plantarflexions, Figure 2A & 4). In contrast, there always remains a certain level of intracortical inhibition in the SOL even when this muscle acts as agonist. Thus, for the TA, the motor cortex can adjust its activation very precisely when acting as agonist or antagonist, while for the SOL, the modulation of inhibition

Journal of Experimental Biology • Accepted manuscript

is much weaker (please also see results from control experiment in the antagonistic muscles, Figure 4). This is further supported by the difference in the duration of the silent period between the two muscles. During the plantarflexions, the CSP was significantly longer in the SOL compared with the TA during the dorsiflexions. It is commonly believed that SICI (for review, see Di Lazzaro and Rothwell, 2014) as well as the CSP (Di Lazzaro et al., 2002; Fuhr et al., 1991; Inghilleri et al., 1993) are cortical mechanisms but that they are mediated by different classes of GABAergic interneurons. While SICI is believed to involved $GABA_A$ receptor-mediated activity, the CSP depends on the activity of $GABA_B$ interneurons (Di Lazzaro et al., 2006; Teo et al., 2009; Ziemann, 2013). As SICI was greater and the CSP duration longer in the SOL than in the TA when both muscles acted as agonists, it seems that there exists a rather general difference in inhibitory control of the two muscles involving the activity of $GABA_A$ as well as $GABA_B$ interneurons. Thus, task-specific modulation of inhibition seems to be less well adapted in the SOL than in the TA, as both SICI and SP are less reduced when the SOL acts as agonist. Nevertheless, we cannot rule out spinal contributions to the CSP, as it was shown that spinal inhibitory networks can contribute to the CSP (Yacyshyn et al., 2016).

Limitations

In the present study, the unconditioned test MEP size differed significantly between the active and the resting conditions. While there is research that showed that the size of SICI can be affected by the size of the unconditioned test MEP (Daskalakis et al., 2002; Sanger et al., 2001; Wagle-Shukla et al., 2009), there is also evidence that the stimulation intensity rather than the size of the unconditioned test MEP affects SICI (Garry and Thomson, 2009; Zoghi and Nordstrom, 2007). Therefore, it was decided that the best solution is to determine the active and resting thresholds for each condition and to adjust the stimulation intensity for each muscle and each condition individually, as we believe that this is the best compromise that can be achieved. Thus, we believe that the comparison between the rest and the active condition is a valid claim, even though it cannot be ruled out that the differences in the size of the unconditioned test MEP affected the size of SICI. Furthermore, the data reported in the antagonist muscles of the main experiment was not collected under ideal conditions, as the position of coil was established for the agonist and not for the antagonist. In order to counteract this limitation, the control experiment was performed where the coil position and the TMS intensity were adjusted for each muscle (SOL, TA), condition (plantarflexion, dorsiflexion), and also whether the two muscles were acting as agonists or antagonists. The results of the control experiment show the same pattern of how SICI was modulated in the SOL and TA when

Journal of Experimental Biology • Accepted manuscript

acting as agonist or antagonist as reported in the main experiment (Figure 4). Thus, all experiments point to the fact that the range of how SICI can be regulated in the TA is much broader and more specific (i.e. low levels of SICI when functioning as agonist vs. high levels of SICI when being antagonist) than in the SOL.

Finally, it is important to note that during plantarflexion as well as dorsiflexion, other muscles than the SOL and the TA very likely contributed to the generation of torque. For example, the triceps surae being the main actuator during plantarflexion, is comprised of the monoarticular SOL and the biarticular heads of the medial gastrocnemius (MG) and lateral gastrocnemius (LG). Even though these muscles are classically referred to as synergists, their activation profile can differ substantially. It was, for example, shown during ramp and hold contractions that the recruitment threshold for LG motor neurons was 20-35 times higher than in the SOL, while the MG motor units displayed higher recruitment thresholds and a more variable firing behaviour than the SOL (Héroux et al., 2014). Even though these findings indicate differences in neural drive during isometric contractions, the role of the motor cortex remains elusive. Furthermore, whether a similar pattern holds true for ballistic types is unclear and subject to future studies. It might be the case that, even though speculative, the cortical control of these muscles differs from that observed in the present study.

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Disclosures

The authors have no conflicts of interest.

Journal of Experimental Biology • Accepted manuscript

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Figures

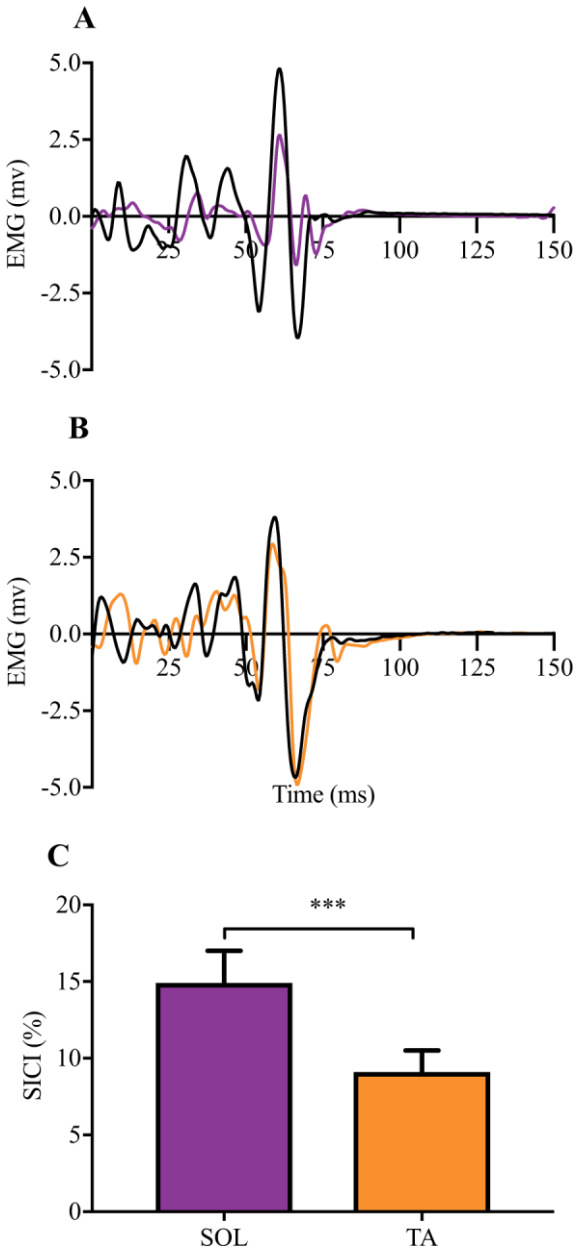

Figure 1:
Representative responses to the TMS stimulation A: in the SOL in one subject during plantarflexion, B: in the TA during dorsiflexion of the ankle and C: the group mean of SICI when the two muscles were acting as agonists. The waveforms display the MEPs after the unconditioned control pulse (black line) and the conditioned pulse (coloured line) in one subject. It can be seen that when both muscles act as agonists, the SOL reveals a considerably greater amount of inhibition. **C:** SICI in the SOL and TA when acting as agonists, i.e. the amount of SICI in the SOL was therefore assessed during plantarflexions, whereas SICI in the TA was measured during dorsiflexions. It can be seen that SICI was significantly lower in the TA (***p = 0.001).

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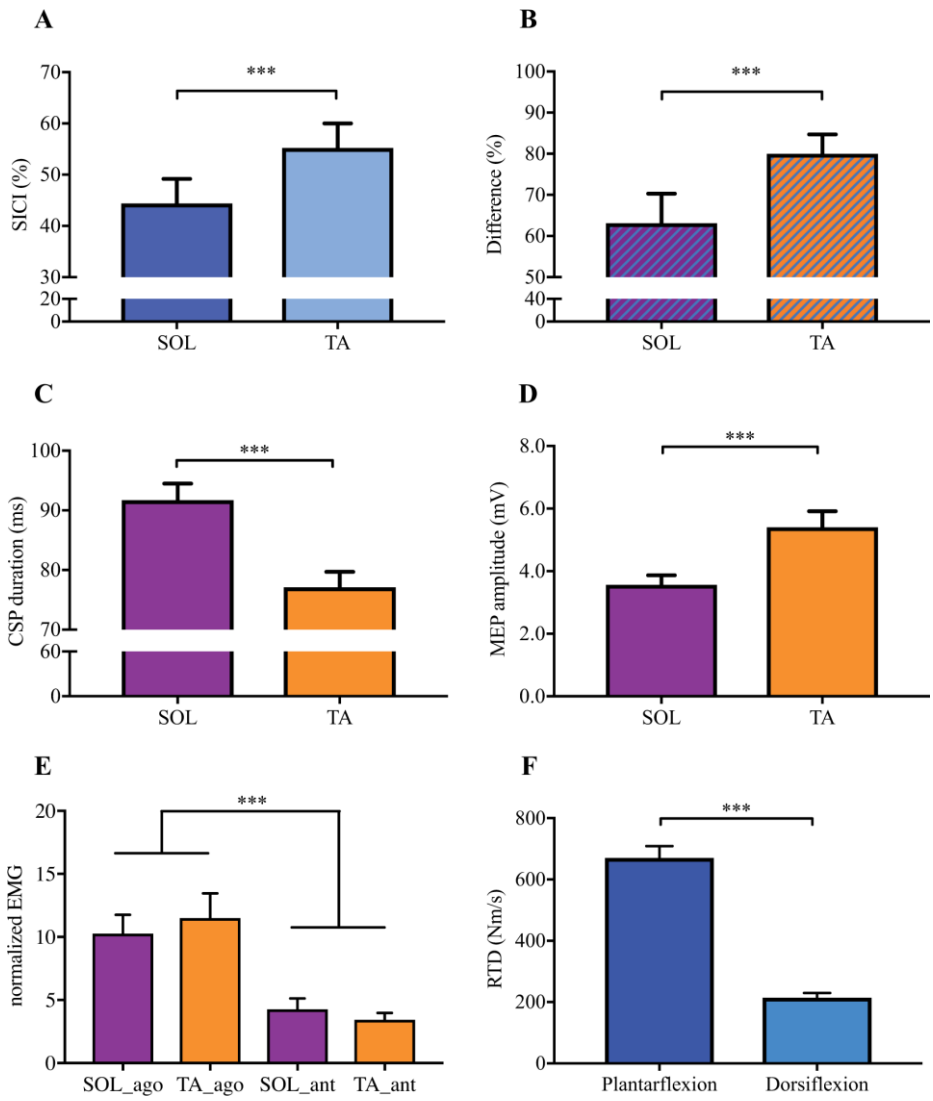

Figure 2:
Overview of the electrophysiological results: A: Amount of intracortical inhibition during rest. SICI was significantly higher in the TA (***p = 0.001) compared with the SOL. **B:** Differences in SICI between the rest and the active condition. To estimate the range of how SICI can be modulated from the active to the resting condition, the percentage difference between these two conditions was calculated. The range in SICI modulation was significantly higher (***p= 0.001) in the TA compared with the SOL. **C:** Cortical silent periods of the SOL and the TA. The duration of the cortical silent period (CSP) during the active condition was significantly (***p = 0.001) longer in the SOL during the plantarflexions compared with the TA during the dorsiflexions. **D:** Peak-to-peak MEP amplitude**.** The peak-to-peak amplitude of the control MEP (single-pulse stimulation) during the plantarflexions (SOL) was significantly smaller compared with the MEP during the dorsiflexions in the TA. **E:** Background EMG. There was no difference in the bEMG in the SOL and TA when acting as agonists or when they were antagonists, but there was a significant difference between the agonist (ago) or antagonist (ant) with higher levels of activation when being ant (***p = 0.005). **F:** Differences in the RTD between the SOL and the TA**.** The rate of torque development measured during the isometric contractions was significantly greater during the plantarflexions compared with the dorsiflexions (***p = 0.001).

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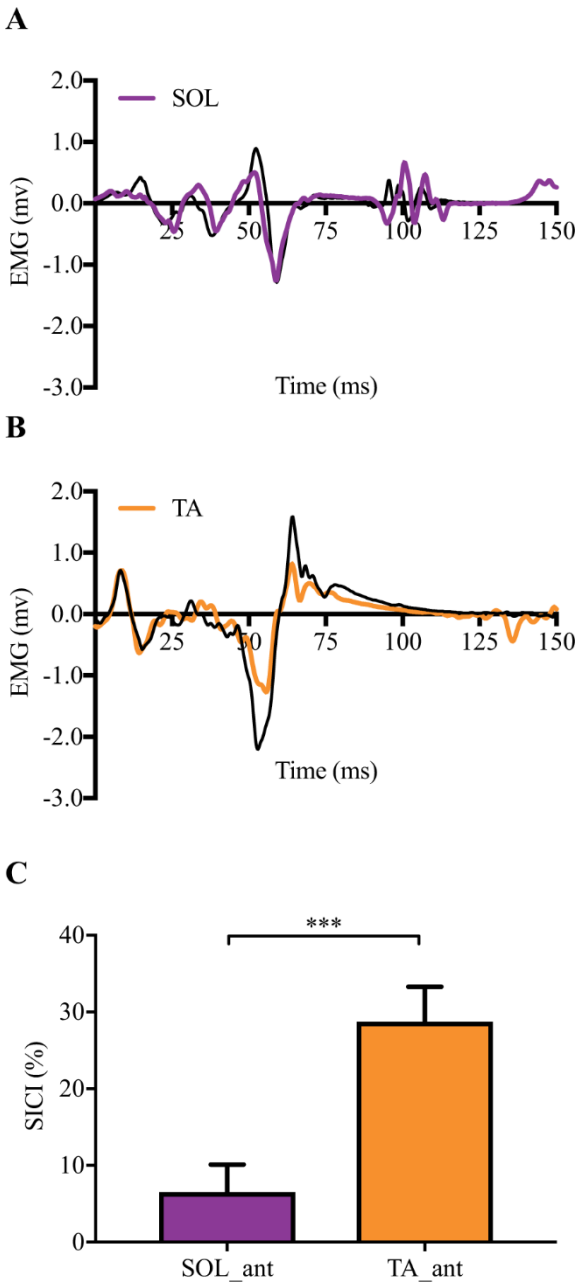

Figure 3:
Representative responses to the TMS stimulation in one subject in the SOL (A) when acting as antagonist during dorsiflexion and the TA (B) during plantarflexions of the ankle as well as the group mean of SICI when the two muscles were acting as antagonists (C). The waveforms display the MEPs after the unconditioned control pulse (black line) and the conditioned pulse (coloured line) in one subject. **C:** SICI in the SOL and TA when acting as antagonists, i.e. the amount of SICI in the SOL was therefore assessed during dorsiflexions, whereas SICI in the TA was measured during plantarflexions. It can be seen that SICI was significantly greater in the TA (***p = 0.001) compared with the SOL.

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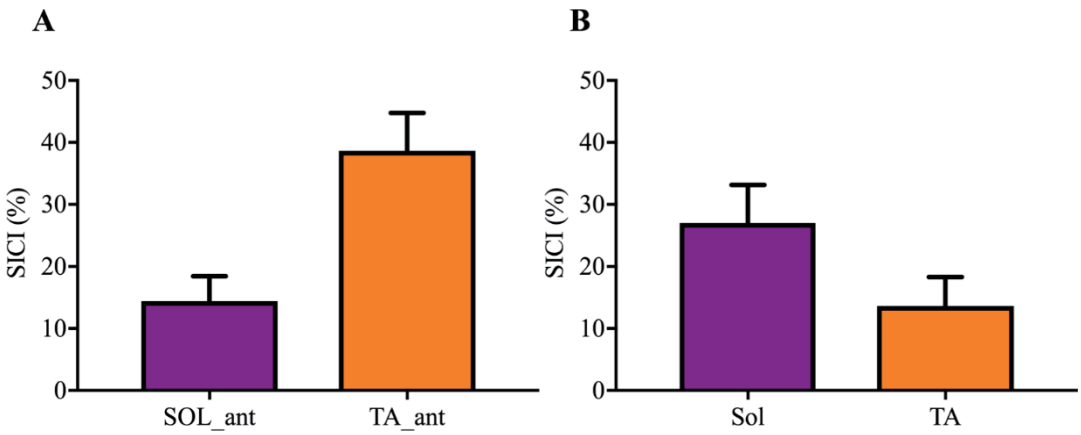

Figure 4:
Results from the control experiment: A: Amount of SICI when the SOL and TA acted as antagonists with higher SICI values in the TA than in the SOL. **B:** Levels of SICI in the SOL and TA when acting as agonist. There was a much higher level of SICI in the SOL compared with the TA. Thus, the results of the control experiment resemble the ones from the main experiment. There was a significant difference in SICI in the TA between being agonist vs. antagonist, which was not the case for the SOL.

Journal of Experimental Biology • Accepted manuscript