厚生労働科学研究費補助金
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脊髄損傷後の歩行機能回復のための
新たなニューロリハビリテーション方法の開発に関する研究

平成21年度　総括・分担研究報告書

研究代表者　赤居　正美

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総括研究報告書

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研究要旨

本研究では、歩行機能再獲得の鍵と目される、脊髄パターン発生器（central pattern generator: CPG）の性質に着目し、その活動を励起させる神経生理学的機序を検証する。それを基に新たな神経リハビリテーション方法の構築をめざす。
近年、特に欧米を中心に、体重を部分的に免荷した状況下で下肢の動作を補助し、正常な歩容を再現する「免荷式歩行トレーニング」が、歩行リハビリテーションの主流となりつつある。この動向には、近年の神経科学領域の研究によって脊髄組織を含む中枢神経系が可塑的性質を持つことが明らかにされ、繰り返しの歩行訓練によって,かなりの歩行機能再獲得が実現できる可能性が示されたことが大きく影響している。
最近報告された免荷式歩行トレーニングの効果を検証する大規模な無作為化比較試験では、ASIA CおよびDに分類される脊髄不全損傷者のうち、９割にトレーニングによる歩行能力の改善が認められている。しかし一方で、従来の歩行リハビリテーションとの比較では、その効果に統計的有意差は認められず、どのような戦略を採ればより効果的な歩行機能再獲得が実現できるのか、あるいは障害や麻痺状態などによってどの程度までの機能回復を見込めるのか、については未だ不明な部分が多い。また、免荷式歩行トレーニングの効果を検証した幾つかの研究では、介入後の歩行速度や動作の改善、運動中の筋活動の改善などの効果を報告しているものの、歩行機能改善の具体的な神経機序については、明らかにされていない。
具体的には、１年目に脊髄損傷者の残存神経機能の定量的把握と、歩行運動出力を高めるための具体的な方策を検討し、その成果に基づいて、２年目に歩行リハビリテーションプログラムを作成する。最終年度には、開発したリハビリプログラムの評価・改善を行うとともに、歩行リハビリテーションによる健康・体力、ADL、社会生活への波及的効果についても包括的に検討する。
本研究の成果は、脊髄損傷者の移動能力の維持・向上を図る、より効果的な新たな神経リハビリテーション（neurorehabilitation）法を立案する上で極めて有用な情報を提供し得るものと考えられる。

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中澤　公孝
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飛松　好子
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A．研究目的

体重免荷によるトレッドミル歩行は「正常な歩行動作を再現することにより種々の求心性感覚入力を脊髄CPGに与え、その活動を改善する」という理論的基盤をもつ。しかし、実際のところ歩行機能の再獲得に至る神経生理学的機序は未だ明らかではない。本研究では、歩行機能再獲得の鍵と目される、脊髄CPGの性質に着目し、その活動を励起させる神経生理学的機序を検証し、それを基にした新たな神経リハビリテーション方法の提案を目指す。
すなわち、本研究の目的は脊髄CPGの活動を励起する適切な神経入力を与えることにより可塑的変化を促し、合目的的に歩行機能回復を実現する神経リハビリテーション方法を開発することである。

B．研究方法

脊髄CPGの特性と歩行運動出力を促進させるための具体的方策の検討を行う。歩行運動の発現に重要な役割を果たすとされる脊髄CPGの特性について検討する。歩行に関連した繰り返しの感覚情報の入力は、脊髄神経回路の適応変化を引き起こすために極めて重要である。本研究では、麻痺領域に発現する歩行様筋活動の変化に着目し、各種感覚情報（荷重、股関節求心系など）との関連、重畳的な随意性神経指令や異なる体肢からの神経情報との関連を詳細に検討する。さらにその成果をもとに、脊髄CPGの活動を励起するための方法を検索する。具体的には、動力型歩行補助装置（Lokomat）による外的な歩行キネマティクスの形成を軸として、歩行運動出力を促通すると考えられる種々の末梢性感覚情報、異なる体肢

からの神経情報、付加的な電気刺激による感覚入力、脳からの重畳的な随意神経指令を組みあわせた新たな方法論を検討する。今年度の研究成果は、２年目以降の歩行機能回復のための神経リハビリテーションプログラムの開発・評価の理論的根拠を担う。

（倫理面への配慮）
研究は国立障害者リハビリテーションセンター倫理審査委員会の承認を得た上で実施する（取得済）
被験者には事前に研究趣旨を文書で説明し、同意を得る。研究で得たデータの公表にあたっては、個人を特定できないように配慮するなどプライバシーの尊重に特段の配慮をする。また研究で得たデータを被験者に伝え、健康管理に有効に役立てる方法を考慮する。

Ｃ．研究結果
Lokomatを用いて脊髄不全損傷（不全対麻痺）患者のトレーニング実験を行い、介入効果の評価法としての経頭蓋磁気刺激(transcranial magnetic stimulation; TMS)と下肢筋の誘発運動電位(motor evoked potential; MEP)、とりわけ前脛骨筋との関連性を見ることが出来た。また足底を中心とした荷重情報の有無や上肢運動の付加、被験者の集中力などの、いいかえればactive assistive trainingの重要性が証明された。

Ｄ．考察
近年の神経科学領域の研究の進展によって、運動機能障害者の歩行機能獲得のための新たなリハビリテーション方法が注目されている。しかし、免荷式歩行トレーニングを含む新たな歩行リハビリテーションは、専門的な知識と高度に習熟した技術が必要である。訓練効果がセラピストの技術に依存することは、広くリハビリテーション方法を普及させる際には大きな弊害となる。
本研究で用いるLokomatは簡便な操作方法のもと、対象を選ばず正常な歩行動作の再現が可能である。本研究を通して歩行機能再獲得の神経機序の解明と、効果的に歩行再獲得を促す方法が開発されれば、多くの運動機能障害者が効果的な歩行リハビリテーションを行う環境を整備することが可能となるものと考えられる。また、本研究はリハビリテーション方法の考案と実践、その結果に基づいた評価と改良を行う包括的な研究である。これらの研究を通して、脊髄損傷者の歩行機能再獲得のために具体的な方法論の提示が可能となる。

Ｅ．結論
３年計画の初年度として、皮質脊髄路を中心とした神経生理機能の定量的把握と介入手法の絞り込みを行った。

Ｆ．健康危険情報
特になし

Ｇ．研究発表
1.　論文発表

- Ogata H, Higuchi Y, Ogata T, Hoshikawa S, Akai M, Nakazawa K:Pressor response to passive walking-like exercise in spinal cord-injured humans. Clinical Autonomic Research 19:113-122, 2009
- Kamibayashi K, Nakajima T, Takahashi M, Akai M, Nakazawa K:Facilitation of corticospinal excitability in the tibialis anterior muscle during robot-assisted passive stepping in humans. European Journal of Neuroscience 30:100-109, 2009
- Obata H, Kawashima N, Akai M, Nakazawa K, Ohtsuki T:Age-related changes of the stretch reflex excitability in human ankle muscles. Journal of Electromyography and Kinesiology 20:55-60, 2010
- Kamibayashi K, Nakajima T, Akai M, Nakazawa K: Effect of sensory inputs on the soleus H-reflex amplitude during robotic passive stepping in humans. Experimental Brain Research 202:385-395, 2010

2.　学会発表
（発表誌名巻号・頁・発行年等も記入）

Ｈ．知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
無
2. 実用新案登録
無
3. その他

厚生労働科学研究費補助金（障害保健福祉総合研究事業）
分担研究報告書

脊髄損傷後歩行機能再獲得のための神経リハビリテーション方法の開発

研究分担者　　中澤　公孝　　東京大学大学院総合文化研究科　教授

研究要旨
近年、神経科学領域で注目されている脊髄可塑性、脊髄CPGの知見を基盤とし、科学的根拠に立脚したあらたな神経リハビリテーション（neurorehabilitation）方法の構築を目指す。本研究では、脊髄損傷者が潜在的に持っている残存歩行能力を最大限に引き出し、健康増進とＡＤＬ向上を導くためのリハビリテーションプログラムを開発する。

Ａ．研究目的

体重免荷によるトレッドミル歩行は「正常な歩行動作を再現することにより種々の求心性感覚入力を脊髄CPGに与え、その活動を改善する」という理論的基盤をもつ。しかし、実際のところ歩行機能の再獲得に至る神経生理学的機序は未だ明らかではない。本研究では、歩行機能再獲得の鍵と目される、脊髄ＣPGの性質に着目し、その活動を励起させる神経生理学的機序を検証し、それを基にした新たな神経リハビリテーション方法の提案を目指す。すなわち、本研究の目的は脊髄CPGの活動を励起する適切な神経入力を与えることにより可塑的変化を促し、合目的的に歩行機能回復を実現する神経リハビリテーション方法を開発することである。

Ｂ．研究方法

脊髄不全損傷者１０名を対象として、動力歩行訓練装置Lokomatによる受動歩行を実施した際に下肢に発現する「歩行様筋活動」が、荷重情報や関節可動域の変化に伴う求心性感覚情報に応じてどのように変化するのかを検討した。また、歩行運動出力の発現に対する上肢運動の貢献度を検討するために、下肢の受動運動に上肢の運動を付加した時の歩行様筋活動の変化を検討した（図１）。

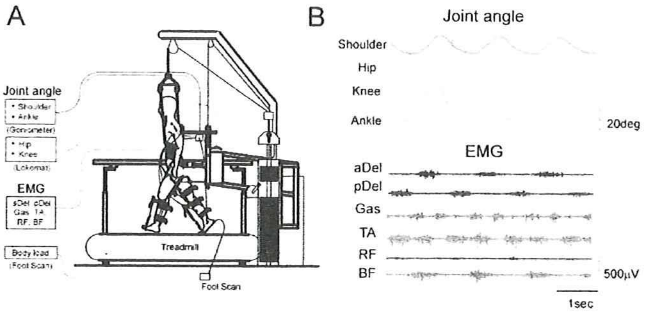


図1　本年度実施した実験の模式図

Ｃ．研究結果

受動歩行運動中には下肢に歩行運動周期に同調した筋活動が認められ、腕振り運動の有無に応じてその振幅と活動位相が変化する結果が得られた。具体的には、腕振り運動および随意指令の付加によって前脛骨の活動位相が短縮し、逆にヒラメ筋の活動が増加した。この変化は付加的な随意指令を与えることでより顕著となる結果が得られた。

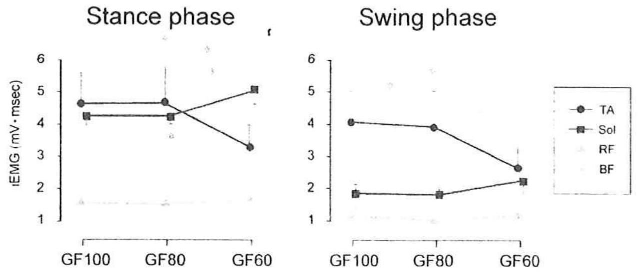


図2　受動運動中に随意指令を与えた際の歩行様筋活動の変化。
GF100は受動歩行、GF60は歩行動作の60%を装置がアシストした状態。
TA：前脛骨筋　Sol：ヒラメ筋　RF：大腿直筋　BF：大腿二頭筋

本年度の実験の結果、荷重情報の増加が歩行様筋活動を増加させること、上肢運動の印加が下腿屈筋（前脛骨筋）の活動位相を変化させることが明らかにされた。脊髄不全損傷者の場合、下肢受動運動のみの場合は歩行時の正常な筋活動位相とはことなり、前脛骨筋の活動が遊脚期のみならず立脚期でも認められるが、このabnormalな活動は、上肢運動の印加により減弱し（TA）、さらに随意神経指令によって正常に近い活動位相を示すことが明らかにされた。興味深いことに、拮抗筋であるヒラメ筋の活動は、前脛骨筋とは逆に上肢運動の印加、随意神経指令に伴って増加する傾向が認められた（図２）。これらの結果は、上肢の運動が下肢の歩行運動出力を、単に増大させるだけでなく、適切に調節している事実を支持するとともに、歩行機能回復のためのリハビリテーション方法考案の貴重な資料となるものと考えられる。

Ｄ．健康危険情報

特になし

Ｅ．研究発表

1.　論文発表　なし
2.　学会発表
・佐藤尭彦他　ヒトの歩行運動における腕振り動作の役割　第16回生体生理工学シンポジウム（大阪）：2004.11

Ｆ．知的財産権の出願・登録状況

1.　特許取得
　特になし
2.　実用新案登録
　特になし
3.　その他

研究成果の刊行に関する一覧表

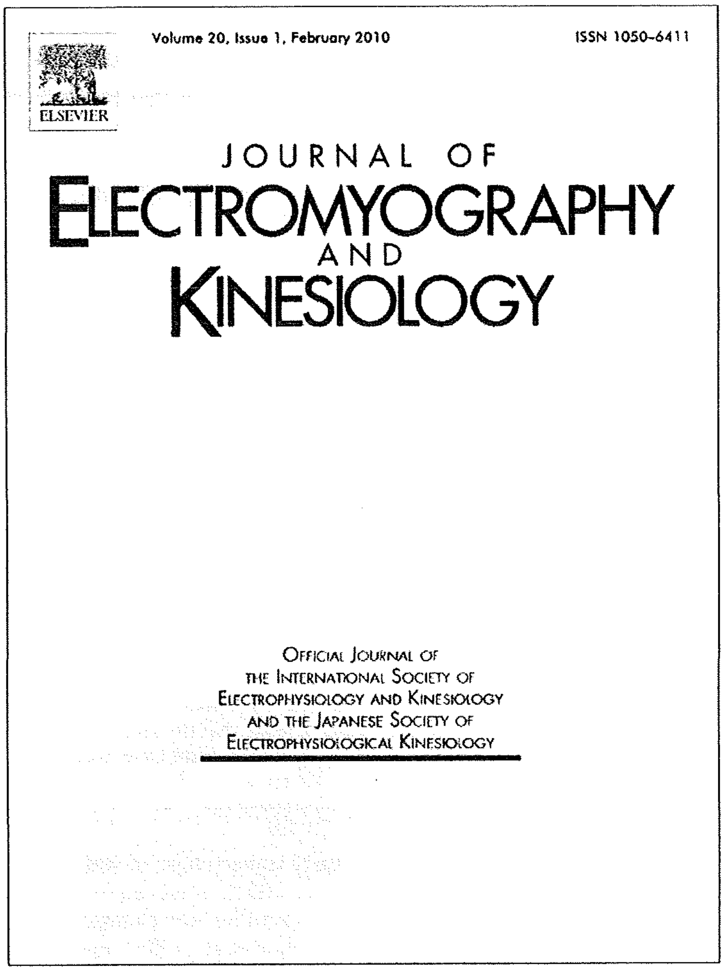
書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書 籍 名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Obata H, Kawashima N, Akai M, Nakazawa K, Ohtsuki T	Age-related changes of the stretch reflex excitability in human ankle muscles.	Journal of Electromyography and Kinesiology	20	55-60	2010
Kamibayashi K, Nakajima T, Takahashi M, Akai M, Nakazawa K	Facilitation of corticospinal excitability in the tibialis anterior muscle during robot-assisted passive stepping in humans.	European Journal of Neuroscience	30	100-109	2009
Kamibayashi K, Nakajima T, Akai M, Nakazawa K	Effect of sensory inputs on the soleus H-reflex amplitude during robotic passive stepping in humans.	Experimental Brain Research	202	385-395	2010
Ogata H, Higuchi Y, Ogata T, Hoshikawa S, Akai M, Nakazawa K	Pressor response to passive walking-like exercise in spinal cord-injured humans.	Clinical Autonomic Research	19	113-122	2009

研究成果の刊行物



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Age-related changes of the stretch reflex excitability in human ankle muscles

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ABSTRACT

The purpose of this study was to characterize the effects of aging on the stretch reflex in the ankle muscles, and in particular to compare the effects on the ankle dorsi-flexor (tibialis anterior: TA) and the plantar-flexor (soleus: SOL). Stretch reflex responses were elicited in the TA and SOL at rest and during weak voluntary contractions in 20 elderly and 23 young volunteers. The results indicated that, in the TA muscle, the elderly group had a remarkably larger long-latency reflex (LLR), whereas no aging effect was found in the short latency reflex (SLR). These results were very different from those in the SOL muscle, which showed significant aging effects in the SLR and medium latency reflex (MLR), but not in the LLR. Given the fact that the LLR of the TA stretch reflex includes the cortical pathway, it is probable that the effects of aging on the TA stretch reflex involve alterations not only at the spinal level but also at the cortical level. The present results indicate that the stretch reflexes of each of the ankle antagonistic muscles are affected differently by aging, which might have relevance to the neural properties of each muscle.

1. Introduction

It is well recognized that aging affects the performance of motor tasks, such as the maintenance of posture and balance control (Bohannon et al., 1984; Horak et al., 1989). It is presumed that both the central and peripheral nervous systems contribute to these phenomena, but the precise mechanisms are still unknown (Stelmach et al., 1989). Stretch reflex, a simple neural circuit that responds to the sudden stretch of a muscle, might be also affected by aging.

Some investigations have paid attention to the effect of aging on the spinal reflex modulation of the soleus (SOL) muscle, which is a key muscle for postural control and bipedal walking (Koceja et al., 1995; Angulo-Kinzler et al., 1998; Chalmers and Knutzen, 2002; Kawashima et al., 2004). We previously demonstrated that elderly subjects show augmented short stretch reflex response in the SOL muscle at rest as compared to those in young subjects (Kawashima et al., 2004). It has also been demonstrated that elderly subjects lack the ability to modulate the SOL motoneuronal excitability for instance, Koceja et al. (1995) showed abnormal modulation of the motoneuronal excitability by postural changes.

On the other hand, recent studies have suggested that the stretch reflex of the tibialis anterior (TA), the antagonistic muscle of the SOL muscle, also has an important role in stabilizing the ankle joint during upright standing (Nakazawa et al., 2003), and the early stance phase of walking (Christensen et al., 2001; Nakazawa et al., 2004). However, to the best of our knowledge, only one study has examined the changes of the TA muscle due to aging. Although Nardone et al. (1995) reported that the amplitude of the reflex action obtained in the TA muscle during upright standing did not depend on age; it remains unknown how the stretch reflex function of the TA muscle changes with age, and whether or not the aging process of this muscle is identical to those in other muscles.

It is well known that each of the SOL and TA muscle have different neuronal characteristics, such as different degrees of connection to the motor cortex (Bawa et al., 2002), and different modulation effects on the spinal reflex excitability (Katz et al., 1988). Interestingly, in contrast to the SOL muscle, the TA stretch reflex shows a larger long-latency reflex (LLR) component, which presumably involves the transcortical pathway (Petersen et al., 1998). Given these facts, it is very likely that the aging process of the stretch reflex in the TA muscle is different from that in the SOL muscle.

The purpose of this study was therefore to characterize the effect of aging on the stretch reflex in the ankle muscles, and in particular how they differ between the ankle dorsi-flexor TA and the plantar-flexor SOL.

2. Methods

2.1. Subjects

Twenty healthy elderly volunteers (mean age 68.0 ± 5.9 years, male = 11, female = 9) and twenty-three young healthy volunteers

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(22.4 ± 1.8 years, male = 11, female = 12) with no history of neurological or muscle disorders participated in the present study. The subjects gave their informed consent to participate in this study, which was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the National Rehabilitation Center for Persons with Disabilities, Tokorozawa, Japan.

2.2. Procedures

Each subject remained seated comfortably in a chair with the right leg fixed to a footplate connected to a servo-controlled torque motor with a rotary encoder (Senoh Inc., Tokyo, Japan). The axis of rotation of the footplate was aligned with the axis of rotation of the ankle joint. The hip and knee angles were fixed at 40° and 50° flexion positions, respectively (anatomical position was 0°). The electromyographic (EMG) activities in the SOL and TA muscles were recorded using bipolar surface electrodes (Ag/AgCl, diameter 7 mm) placed on the muscle belly at an inter-electrode distance (center to center) of 15 mm. The EMG signals were amplified and band-pass-filtered by a bipolar differential amplifier (AB-621B Nihon Kohden, Tokyo, Japan) with low and high cut-off frequencies between 15 and 1000 Hz, respectively. The EMG, torque and angle signals were digitalized at a sampling rate of 1 kHz (WE7251; Yokogawa Electric Co., Tokyo, Japan) and stored in a computer.

Prior to the stretch reflex tests, the subjects were asked to exert the maximal isometric voluntary contraction for the SOL and the TA muscle in turn in order to determine the maximal EMG level. In this manuscript, the term "MVC" indicates the EMG level during maximal isometric voluntary contraction. The subjects were asked to maintain two contraction levels, i.e., rest (condition REST) and weak voluntary contraction (approximately 10% MVC: condition ACT) in the TA for the TA stretch test and in the SOL for the SOL stretch test. To control the contraction level, the smoothed full-wave rectified EMG and the reference line corresponding to 0% and 10% MVC were displayed on an oscilloscope, and the subjects were asked to keep a rectified EMG at a target level. A quick stretching of the TA or the SOL was given by imposing a quick rotation of the ankle joint at a range from 10° plantar-flexion to 5° dorsi-flexion. The direction of the rotation was plantar-flexion for the TA stretch test and dorsi-flexion for the SOL stretch test. Three different angular velocities, approximately 150 (slow: SL), 250 (moderate: MD), and 350°/sec (fast: FS) were applied five times in random order and at random stimulus intervals (10–15 s). The orders of stretching and contraction levels were randomized.

2.3. Data analysis

The digitized EMG signals were full-wave rectified after subtraction of the DC bias. The mean background EMG activity (BGA) level was then subtracted from the evoked EMG responses. The BGA was calculated during the 100 ms prior to the onset of stretches. In the present study, the incidence of reflex elicitation was calculated to show how often the stretch reflex response appeared in each group and for each contraction level and angular velocity. The number of observed responses was expressed as the ratio to the total number of stretches. The criterion of reflex appearance used for the probability was whether an EMG response reached a level higher than the BGA plus three times its standard deviation (BGA + 3SD). Stretch reflex responses were divided into their short- (SLR), middle- (MLR), and LLR components. The onset and the endpoint of the stretch reflex response were defined in the same manner as the probability of reflex elicitation. In accordance with previous studies (Schieppati and Nardone, 1997; Christensen et al., 2001), the onset of MLR was defined as 20 ms after the onset of SLR and that of LLR was defined in as 20 ms after the onset of MLR. Thus, the duration of SLR and MLR was defined as 20 ms, respectively. The duration of LLR was determined from the above mentioned onset to the endpoint of the stretch reflex response. In the present study, the mean amplitude of the rectified EMG with the BGA value subtracted was used to evaluate the size of each stretch reflex component.

The statistical differences of incidences were tested by the χ^2-test at each muscle contraction level and angular velocity. Statistical differences in each reflex component were tested by two-way analysis of variance (ANOVA, 2 × 2, muscle contraction level × group). Scheffé's post-hoc comparisons were used to determine the statistical differences between the REST and ACT conditions and between elderly and young groups. The significance level was set at $P < 0.05$.

3. Results

3.1. Background EMG activity

We confirmed that the level of BGA of the SOL and TA muscles were similar between the elderly and young groups. In the SOL stretch test, the SOL BGA at REST were 0.4 ± 0.07 and 0.4 ± 0.08% MVC for young and elderly, respectively and increased to 9.6 ± 0.59 and 9.9 ± 0.74% MVC at ACT. In the TA stretch test, the TA BGA at REST were 0.4 ± 0.19 and 0.5 ± 0.14% MVC for young and elderly, respectively and increased to 8.6 ± 0.33 and 9.6 ± 0.60% MVC at ACT.

3.2. Probability of reflex elicitation

In the present study, the stretch reflex responses of the TA appeared more frequently in the elderly subjects than in the young subjects. The probability of reflex elicitation in each condition, stretch speed, and muscle is summarized in Table 1. As shown in this table, a stretch reflex response could not be obtained in all subjects. Moreover, the number of responses increased with angular velocity and contraction level. The probability of the TA stretch reflex response in REST was statistically higher in the elderly subjects than in the young subjects at each angular velocity (χ^2-test, $P < 0.05$). On the other hand, the probability of the SOL stretch reflex response in REST tended to be lower in the elderly subjects (χ^2-test, $P < 0.05$). Therefore, we calculated the latency and amplitude of the reflex responses only at the fastest velocity and for subjects who responded to both the REST and ACT conditions in each stretch test.

3.3. Stretch reflex EMG responses

Fig. 1 shows typical waveforms of stretch reflex responses in the TA and the SOL under both postural conditions, REST and ACT, obtained from one subject in each group. As clearly shown in this figure, there was a remarkable difference in the reflex amplitude between the TA and SOL stretch reflex responses. The elderly subjects showed a relatively larger (Fig. 2) and longer response (Table 2) in both REST and ACT in the TA.

3.4. Latency and duration of the stretch reflex EMG responses

The onset of the TA stretch reflex response was earlier in the ACT than in the REST ($F_{(1, 32)} = 15.8$, $P < 0.01$), while no difference was found in latency between the two subject groups (Table 2). In contrast, there was no effect of the contraction level in the SOL stretch reflex response, whereas the effect of group was significant ($F_{(1, 32)} = 23.1$, $P < 0.01$) in this muscle.

An interaction between groups and contraction levels was found in the durations of the TA stretch reflex ($F_{(1, 32)} = 16.0$,

Table 1
The probability of reflex elicitation at each angular velocity for the (a) TA and (b) SOL stretch tests. *Significant difference ($P < 0.05$).

(a) TA

	Slow	Moderate	Fast
Elderly			
REST	39.0% (10/20)	57.0% (13/20)	71.0% (19/20)
ACT	58.0% (16/20)	75.0% (20/20)	81.0% (20/20)
Young	*	*	*
REST	19.1% (5/23)	31.3% (12/23)	36.5% (14/23)
ACT	53.0% (15/23)	77.4% (22/23)	80.9% (22/23)

(b) SOL

	Slow	Moderate	Fast
Elderly			
REST	84.0% (17/20)	85.0% (18/20)	88.0% (18/20)
ACT	92.0% (20/20)	98.0% (20/20)	100.0% (20/20)
Young	*	*	*
REST	99.1% (23/23)	98.3% (23/23)	99.1% (23/23)
ACT	98.3% (23/23)	100.0% (23/23)	100.0% (23/23)

Percentage of number of responses to number of stretches (number of responding subjects/total number of subjects)

$P < 0.01$). The duration of the TA stretch reflex was longer in ACT than in REST only in the young subjects ($P < 0.05$). On the other hand, there was no effect of group or contraction level in the SOL.

3.5. Stretch reflex components

Summarized data for each of three reflex components (SLR, MLR, and LLR) are shown in Fig. 2. Differences in the effects of aging between the TA and SOL muscles were mainly found in LLR. The group effect was significant only in LLR of the TA stretch reflex ($F_{(1,32)} = 4.7$, $P < 0.05$): the elderly subjects had larger LLR responses than the young subjects in both contraction levels ($P < 0.05$).

Another difference was found in the SOL, that is, the elderly subjects lacked a significant augmentation in the reflex response size according to the muscle contraction level in SLR and MLR. The augmentation was found only in the young subjects in SLR and MLR ($P < 0.05$). On the other hand, the effect of muscle contraction was significant in all three reflex components in the TA (SLR: $F_{(1,32)} = 42.5$, MLR: $F_{(1,32)} = 20.6$, LLR: $F_{(1,32)} = 7.7$, $P < 0.05$): each component in both groups was larger in ACT than in REST.

The most remarkable age-related differences were found in the LLR component of the TA muscle, and there were distinct differences in the relative EMG magnitudes of the SLR and LLR components between the SOL and TA muscle. Fig. 3 shows the size of the LLR relative to the SLR component. It is clear that the LLR component is larger in the TA muscle, especially in the elderly group. Two-way ANOVA (muscle contraction level × group) revealed a significant main effect of "group" in the TA LLR ($F_{(1,57)} = 13.12$, $P < 0.01$). A post-hoc test showed statistically significant differences between the elderly and young groups under the REST and ACT conditions in the TA LLR.

4. Discussion

The purpose of the present study was to examine the effect of aging on the stretch reflex in the ankle muscles. Our interest was whether the aging process of the ankle dorsi-flexor TA is identical to that in the ankle plantar-flexor SOL. We characterized the differences in the effects of aging on these muscles by observing not only the amplitude of each reflex component and the reflex latency, but also the incidence of the stretch reflex responses when perturbations were applied. The present results clearly demonstrated the following four differences in the elderly subjects in comparison to the young subjects, namely, (1) higher probability of reflex elicitation, (2) longer duration of the reflex response in the TA at REST, (3) larger LLR in the TA at both contraction levels, and (4) lack of contraction level-related augmentation of SLR and MLR in the SOL.

These results suggest that the effect of aging on the stretch reflex is different between the TA and SOL muscles. In the following section, the possible neurological mechanisms underlying these results are discussed.

4.1. Effects of aging on the short and medium stretch reflex component

As clearly shown in the data obtained from the young subjects, the SLR and MLR in both the TA and SOL muscles showed marked increases from the REST to ACT conditions. This facilitated stretch reflex response, called automatic gain compensation, is regarded as an important function to adjust the reflex excitability in accordance with the pre-activation level of each muscle (Matthews, 1986). It can be simply explained by the prior recruitment of more motor units or enhanced sensitivity of the muscle spindle due to α–γ linkage. However, in the elderly subjects, the SLR and MLR of the SOL muscle showed no significant changes between the REST and ACT conditions, although the level of BGA was very different. This result is consistent with the previous results (Kawashima et al., 2004; Chung et al., 2005). In our previous study, we suggested that histochemical alterations in muscle fibers accompanying the

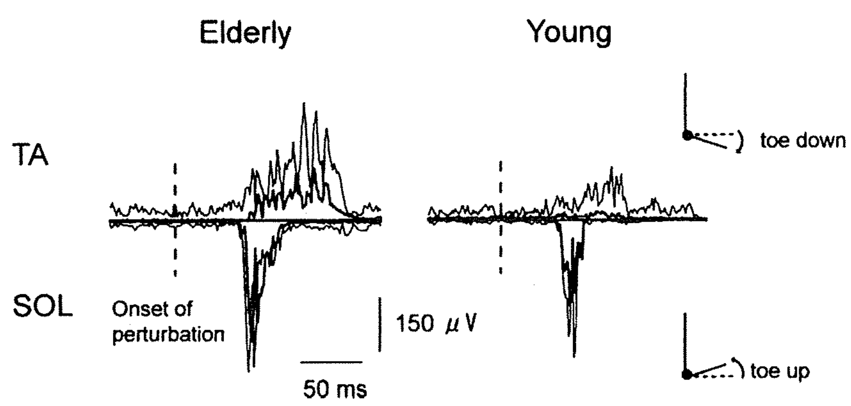

Fig. 1. Typical waveforms of the stretch reflex responses in the TA and the SOL at moderate velocity. The thick line indicates the EMG responses under the REST condition, and the thin line, under the ACT condition. The vertical dotted line indicates the onset of stretch.

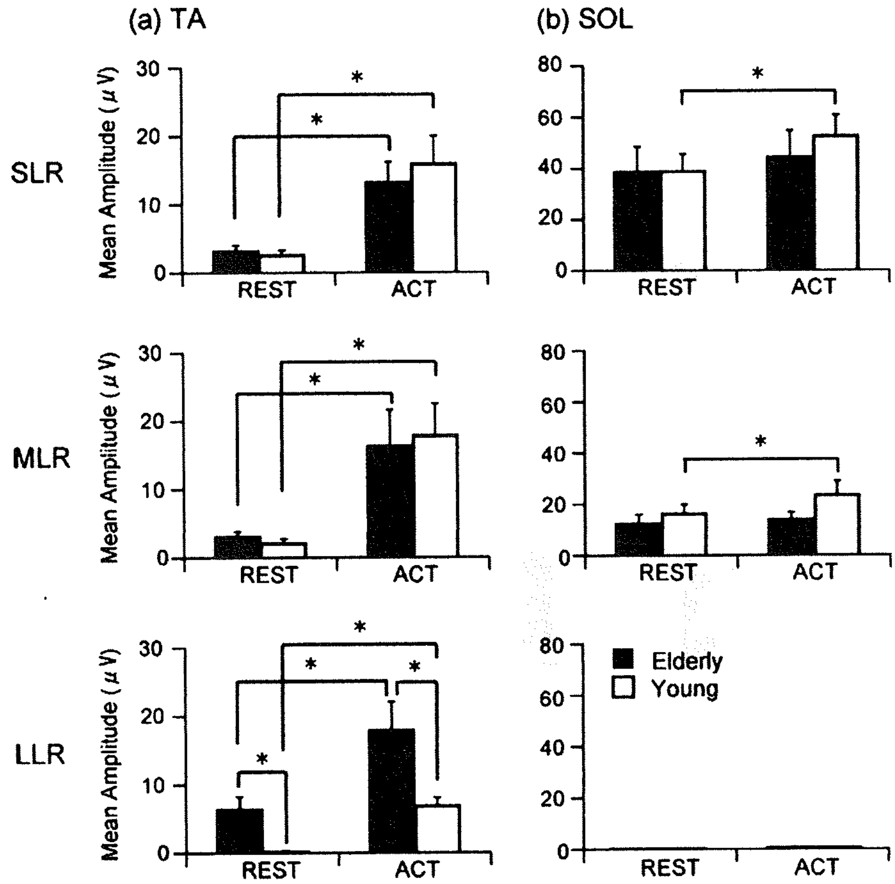

Fig. 2. Comparisons of the mean amplitude of each component between the elderly and young groups and REST and ACT conditions in the (a) TA and (b) SOL stretch reflex responses. The error bars indicate the SE of the mean value. *Significant difference ($P < 0.05$).

Table 2
Means ± SE for latency and duration of the stretch reflex responses in the (a) TA and (b) SOL stretch reflex responses. *Significant difference ($P < 0.05$).

(a) TA

	Latency (ms)	Duration (ms)
Older		
REST	76.8 ± 6.6]*	63.2 ± 7.8
ACT	65.6 ± 6.5	65.7 ± 4.2
Younger		*
REST	69.5 ± 6.6]*	42.9 ± 5.1]*
ACT	60.2 ± 5.2	58.0 ± 3.8

(b) SOL

	Latency (ms)	Duration (ms)
Older		
REST	47.2 ± 0.9	33.9 ± 2.2
ACT	47.1 ± 0.7 *	35.7 ± 1.3
Younger	*	
REST	43.6 ± 0.9	34.9 ± 1.9
ACT	42.8 ± 0.7	37.0 ± 1.3

aging process, such as stiff intrafusal fibers, might affect the SLR in the SOL muscle because the excitability of the motor-neuron pool evaluated with the H-reflex was not different in elderly and young subjects (Kawashima et al., 2004). This explanation may also be applicable to the present result.

4.2. Effect of aging on the long-latency reflex component

There has been some dispute regarding the physiological pathways of the LLR. For the distal muscles in the upper extremities, which are more predominantly under cortical control, the LLR is commonly assumed to involve supraspinal mechanisms (Matthews, 1991). In the lower limb, the neural pathway underlying LLR is likely to depend on the muscle. In the SOL muscle, some authors have reported that the spinal pathway is responsible (Tracey et al., 1980; Koceja and Kamen, 1992), whereas others have indicated that it is the transcortical pathway (Taube et al., 2006). In the TA muscle, it is now assumed that the transcortical pathway is largely involved in the LLR response (Petersen et al., 1998; van Doornik et al., 2004). Therefore, the larger LLR response in the TA muscle in the elderly subjects may indicate that the effect of aging on the TA stretch reflex includes the change in the excitability of the motor cortex. Similarly, Lin and Sabbahi (1998) observed larger stretch reflex magnitude of the long-latency responses in the wrist muscles of the elderly group, and they suggested that the age-related changes of the stretch reflex system involve supraspinal mechanisms.

It is noteworthy that the larger magnitude and longer duration of the LLR component in the elderly subjects were found even at REST. This result suggests that elderly persons have a deficit of adequate suppression of the LLR under resting condition. The enhanced LLR in the TA muscle is known in patients with Parkinson's disease (Ridding et al., 1995). Given the fact that intracortical inhibition is reduced with aging (Peinemann et al., 2001) and in patients with Parkinson's disease (Ridding et al., 1995), the inhibitory mechanisms of the motor cortex might have contrib-

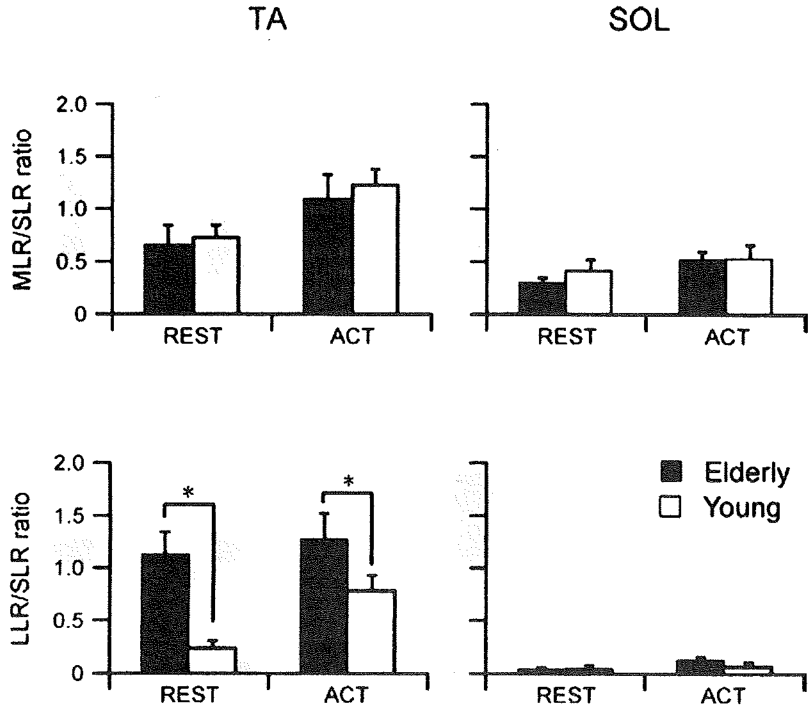

Fig. 3. Comparisons of the amplitude ratios between stretch reflex components between elderly and young groups and REST and ACT conditions in both the TA and the SOL muscles. The error bars indicate the SE of the mean value. *Significant difference ($P < 0.05$).

uted to the present result. Although it is not clear whether or not the same neural mechanism contributes, age-related alteration in the supraspinal mechanism, such as the transcortical feedback loop from the basal ganglia to the motor cortex, may at least in part contribute to the present result.

Some previous studies have investigated the age-related change in the cortico-spinal pathway by using transcranial magnetic stimulation. These studies have shown that greater stimulus intensities are required to reach the same maximal motor output in elderly subjects (Pitcher et al., 2003). Their result suggests that the enhanced LLR component observed in the present study cannot be solely explained by the age-related change in the cortico-spinal pathway. Nevertheless, since the previous investigations have been targeted at the FDI muscle, the involvement of the cortico-spinal pathway cannot be completely ruled out. Alternatively, the enlarged LLR response could be explained by age-related alterations in the peripheral muscle and/or tendon properties, such as the increased half-relaxation time of tendon reflexes previously reported in the elderly (Carel et al., 1979; Koceja et al., 1993). Because detailed information about the mechanism of the larger LLR in the elderly is not available at present, future studies using the TMS technique, such as a short-interval intracortical inhibition or intracortical facilitation will need to be conducted to examine this possibility.

4.3. Different aging process of the stretch reflex between TA and SOL muscles

The present results indicated the occurrence of remarkable age-related changes in the LLR in the TA and SLR and MLR in the SOL. Such differences between the ankle plantar- and dorsi-flexor muscles might be explained by the different neurological and biomechanical features of those muscles. It is well recognized that the connection to the motor cortex is stronger in the TA than in the SOL muscle (Bawa et al., 2002), and that the activity of the ankle dorsi-flexor is more under the control of a supraspinal mechanism (Armstrong, 1988). Dietz indicated in his review article that "the CNS determines the TA response, whereas the SOL response is dominantly modulated by peripheral information" (Dietz, 1992). Moreover, the present result of the TA stretch reflex is similar to that observed in the wrist muscles, in which a larger LLR has been reported with aging (Lin and Sabbahi, 1998). Biomechanical factors are also likely to contribute to the difference. It is well known that the ankle plantar-flexor muscle, but not the ankle dorsi-flexor muscle, acts as an antigravity muscle during standing. Since the ankle plantar-flexor is selectively exposed to body weight for a long time, some peripheral changes are more progressive within the ankle plantar-flexor than they are within the ankle dorsi-flexor.

In conclusion, the present results demonstrated that the effects of aging on the stretch reflex are different in the TA and the SOL. Such differences seem to be related to the different neurological and biomechanical properties of those muscles.

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NEUROSYSTEMS

Facilitation of corticospinal excitability in the tibialis anterior muscle during robot-assisted passive stepping in humans

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Keywords: corticospinal tract, load-related afferent input, locomotion, transcranial magnetic stimulation

Abstract

Although phasic modulation of the corticospinal tract excitability to the lower limb muscles has been observed during normal walking, it is unclear to what extent afferent information induced by walking is related to the modulation. The purpose of this study was to test the corticospinal excitability to the lower limb muscles by using transcranial magnetic stimulation (TMS) and transcranial electrical stimulation of the motor cortex while 13 healthy subjects passively stepped in a robotic driven-gait orthosis. Specifically, to investigate the effect of load-related afferent inputs on the corticospinal excitability during passive stepping, motor evoked potentials (MEPs) in response to the stimulation were compared between two passive stepping conditions: 40% body weight unloading on a treadmill (ground stepping) and 100% body weight unloading in the air (air stepping). In the rectus femoris, biceps femoris and tibialis anterior (TA) muscles, electromyographic activity was not observed throughout the step cycle in either stepping condition. However, the TMS-evoked MEPs of the TA muscle at the early- and late-swing phases as well as at the early-stance phase during ground stepping were significantly larger than those observed during air stepping. The modulation pattern of the transcranial electrical stimulation-evoked MEPs was similar to that of the TMS-evoked MEPs. These results suggest that corticospinal excitability to the TA is facilitated by load-related afferent inputs. Thus, these results might be consistent with the notion that load-related afferent inputs play a significant role during locomotor training for gait disorders.

Introduction

It is well recognized that many patients with incomplete spinal cord injury have a greater chance of regaining walking ability through participating in conventional overground mobility therapy and body-weight-supported treadmill training (Wernig *et al.*, 1995; Barbeau *et al.*, 1999; Wirz *et al.*, 2005; Dobkin *et al.*, 2006). The key concepts of this training are that afferent inputs related to the stepping activate spinal neuronal circuits and that prolonged exposure to locomotor training induces use-dependent plasticity in the central nervous system. Although much attention has been paid to the spinal neural mechanisms, less is known about the changes in corticomotor function that might occur when a patient undergoes the training.

With respect to the corticomotor function during healthy human walking, several transcranial magnetic stimulation (TMS) studies showed modulation in the excitability of the corticospinal tract while walking (Schubert *et al.*, 1997; Petersen *et al.*, 1998, 2001; Capaday *et al.*, 1999; Bonnard *et al.*, 2002). The first of these studies demonstrated that motor evoked potentials (MEPs) of the ankle muscles by TMS were generally modulated in parallel with muscle activation levels during treadmill walking (Schubert *et al.*, 1997). At this point, it is unknown to what extent descending and afferent inputs contribute to this phase-dependent modulation of the corticospinal excitability during walking.

A recently developed driven-gait orthosis (DGO) for locomotor training is capable of drastically reducing the effects of descending commands and of examining the effects of afferent inputs associated with stepping. It has been shown in positron emission tomography and functional MRI experiments that active and passive movements during bicycling (Christensen *et al.*, 2000) and flexion-extension of the wrist joint (Lotze *et al.*, 2003) resulted in a similar pattern of activity in the sensory-motor cortex, although active movement was associated with higher activation in the primary motor cortex. In studies using TMS, modulation in the corticospinal excitability to the forearm muscle has been observed during passive wrist movement (Carson *et al.*, 1999; Lewis *et al.*, 2001; Lewis & Byblow, 2002). From these previous reports, even during passive stepping using the DGO, the corticospinal excitability to the lower limb muscles may be modulated by the peripheral afferent inputs.

The present study was designed to test the changes in the corticospinal excitability to the lower limb muscles by TMS while a healthy subject passively stepped in the DGO. Specifically, as

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peripheral inputs from hip joints and load receptors have been considered important for the generation of locomotor-related muscle activity and for locomotor recovery (Harkema *et al.*, 1997; Dietz & Harkema, 2004), we investigated the effect of load-related afferent inputs on the corticospinal excitability at different body-weight-unloading conditions during passive stepping. By using the same DGO, we have recently observed strong facilitation of the cutaneous reflex in the tibialis anterior (TA) muscle at body-weight-loaded passive stepping but not at completely unloaded passive stepping (Nakajima *et al.*, 2008). Therefore, we hypothesized that the load-related afferent inputs will up-regulate corticospinal excitability to the TA muscle in a phasic manner during passive stepping.

Materials and methods

Subjects

Thirteen healthy subjects (aged 23–44 years) participated in this experiment with the ethical approval of the local ethics committee at the National Rehabilitation Center for Persons with Disabilities. All subjects were included in the main experiment and four of them participated in an additional experiment. None of the subjects had any known history of neurological disorders. Each subject provided informed consent to the experimental procedures, which were in accordance with the Declaration of Helsinki.

Passive stepping

Passive stepping was assisted by a robotic DGO (Lokomat®, Hocoma AG, Switzerland). A detailed description of the device can be found elsewhere (Colombo *et al.*, 2000). The DGO enables the imposition of locomotor stepping without voluntary movements. Briefly, the DGO provides drives for the physiological hip and knee joint movements of each leg. Four separate position controllers control the angles of the hip and knee joints in a computer-based real-time system. The DGO was secured to the subject by straps across the pelvis and chest. The lower limb parts of the orthosis were fixed to the subject with straps around the thigh and shank. Dorsiflexion of the ankle joint for toe clearance during the swing phase was achieved by a passive foot lifter (spring-assisted elastic strap). Additionally, each subject wore an ankle foot orthosis (fixed at 5° dorsiflexion) to prevent large movements in the ankle joint.

To change load-related afferent inputs during stepping, two passive stepping conditions were used, each with a different amount of body weight unloading. One was passive stepping on the treadmill with unloading of 40% body weight (ground stepping), which is close to the level of unloading used in the locomotor training for spinal-cord-injured patients. The other stepping condition was with full (100%) body weight unloading, i.e. the subjects were suspended with the DGO in the air during the passive stepping (air stepping). In both stepping conditions, the stepping speed was set at 1.5 km/h. During passive stepping, the subject was instructed to relax as much as possible and not to prevent the lower limb movements imposed by the DGO.

Measurements

Electromyographic (EMG) activity was recorded from the rectus femoris (RF), biceps femoris (BF), soleus (Sol) and TA muscles in the right lower limb by surface bar electrodes (inter-electrode distance, 10 mm) placed over the muscle bellies. The ground electrode was placed over the malleolus. The EMG signals were amplified (×1000) and band-pass filtered (15–1000 Hz) by a bioamplifier (MEG-6108, Nihon Kohden, Japan). Actual hip and knee joint angles of the orthosis were provided by potentiometers attached to the joint part of the orthosis. The angle of the ankle joint was recorded by an electrogoniometer attached to the anterior aspect of the lower leg and foot (SG110, Biometric Ltd, UK). Ground contact of the heel during ground stepping was detected by a pressure-sensitive sensor placed under the heel (PH-463, DKH, Japan). All signals were sampled at 2 kHz (WE 7000, Yokogawa Co. Ltd, Japan) and stored for later analysis.

Transcranial magnetic stimulation

To investigate the corticospinal excitability in the right lower limb muscles, TMS was applied over the left motor cortex using a magnetic stimulator (Magstim 200, Magstim Co., UK). A double-cone coil (outside diameter, 110 mm) was positioned in the best location for eliciting MEPs in the TA muscle. The center of the coil was placed between the vertex and ~20 mm to the left of the vertex, and current in the coil flowed in the anterior-to-posterior direction. A swimming cap was pulled tightly over the subject's head and the best location for the stimulation was then marked on the cap with white tape. The coil was firmly fixed with a chin band and Velcro® tape and was held by an experimenter. The weight of the coil cable was decreased by suspending it from a support frame that is used to hang up the harness in the body weight support system. The coil position was regularly checked to ensure that no slippage occurred during the experiment.

The stimulus intensity was set to produce a peak-to-peak MEP amplitude of ~0.1 mV in the TA muscle during upright standing with 40% body weight unloading. The MEPs during standing were recorded at least six times. During the ground and air stepping, the TMS stimuli were given at 6-s intervals, slightly out of phase with the step cycle. In this manner, the stimuli were dispersed randomly over the step cycle. In each recording session, 20–30 stimuli were applied in a stepping condition (i.e. each session for approximately 2–3 min). After the rest interval, the other stepping condition was performed; in total, three or four sessions of each stepping condition were conducted.

Additional experiment

Four of the 13 subjects included in the main experiment participated in an additional experiment to compare the pattern of the MEP responses between TMS and transcranial electrical stimulation (TES) during passive stepping. In this experiment, TES was applied using a stimulator (D180, Digitimer Ltd, UK). The time constant of the stimulation pulse was 100 μs. An anode electrode for TES was placed ~20 mm to the left of the vertex and a cathode ~50 mm anterior to the vertex (Di Lazzaro *et al.*, 2001). The stimulation electrodes were firmly secured by a swimming cap. As in the case of TMS, the stimulus intensity for TES was also set to evoke a peak-to-peak MEP amplitude of ~0.1 mV in the TA during upright standing with 40% body weight unloading. After the MEP recording by either TMS or TES was performed under both stepping conditions (ground and air stepping), the MEPs by the other stimulus method were recorded under both stepping conditions. In this additional experiment, the stepping condition and the stimulus timing for TMS and TES were the same as in the main experiment.

Data analysis and statistics

The peak-to-peak MEP amplitudes of TMS and TES were analysed off-line. For each MEP, the peak amplitude in the lower limb muscles was measured within a time window of 20–60 ms after the stimulation. The EMG background activity (BGA) was determined as a root-mean square value of the EMG activity for 50 ms just prior to the stimulation. The joint angle was measured at the instant the stimulation was applied. As there was no heel contact during air stepping, the signal of the heel contact could not be used as a reference point in the step cycle. Therefore, the instant of the maximal flexion of the right hip joint was used as the reference point (i.e. the starting point of a step cycle). The step cycle of the passive stepping was divided into six phases of equal length. For each subject, more than six peak amplitudes were averaged for each phase of the step cycle in each stepping condition. The data are shown as mean ± SEM across subjects in each condition. In the main experiment, the MEPs and BGAs were analysed using a two-way repeated-measure ANOVA with factors of task (ground and air stepping) and phase (six phases in the step cycle). In addition, one-way ANOVA tests were conducted to determine the significant differences under each stepping condition, with the factor of step phase. When the assumption of sphericity by Mauchly's test was violated, Greenhouse-Geisser adjustments to the degrees of freedom were made. When statistical significance was found using the ANOVAS, *post-hoc* tests (Bonferroni) for multiple comparisons were performed to identify significant differences. The significance level was set at $P < 0.05$ in all cases.

Results

Patterns of leg muscle activity and joint movement during passive stepping

Figure 1 shows a typical example of the EMG activities and joint movements under both passive stepping conditions with the DGO. The movement trajectories of the hip and knee joints were similar between ground and air stepping because the limb position was controlled by the computer-based real-time system. The motion of the ankle joint at the stance phase of ground stepping was also similar to that of air stepping because the ankle position was fixed by the foot orthosis. Across the step cycle under both stepping conditions, EMG activity was not observed in the RF, BF or TA muscle. Low EMG activity was seen in the Sol muscle at the stance phase of ground stepping in some subjects.

Transcranial magnetic stimulation-evoked responses in the standing and stepping conditions

During stepping, the TMS was applied at a constant intensity. As there was little or no EMG activity during stepping, the MEPs were detected easily, even at responses as small as ~50 μV. In Fig. 1, the MEPs evoked at the end of the swing phase during ground stepping (the area enclosed by the dotted line) are enlarged in the traces at the right of the figure.

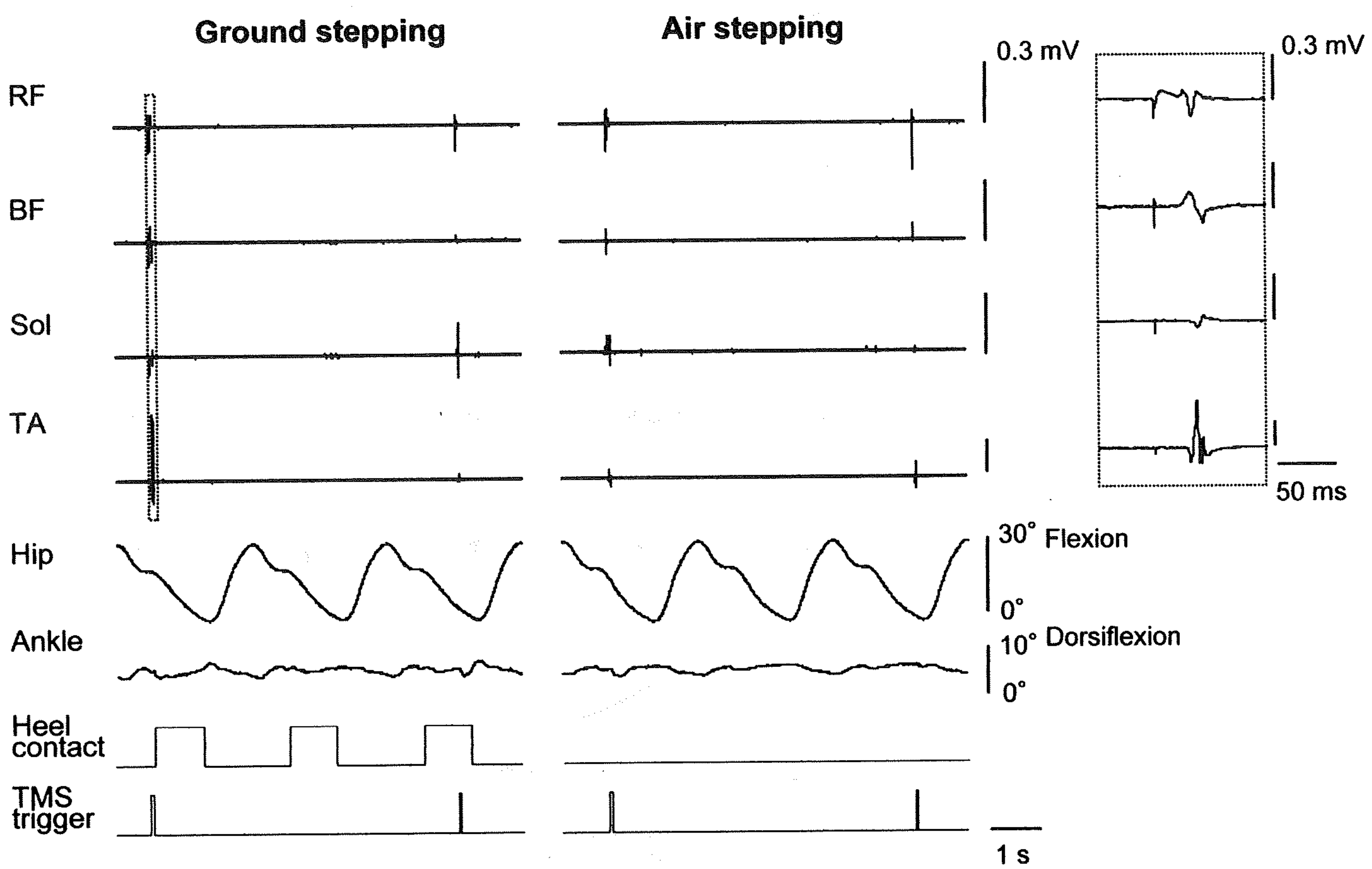

FIG. 1. Example of the raw EMG activities of the RF, BF, Sol and TA muscles and movement trajectories of the hip and ankle joints observed in the right leg during ground and air stepping. The heel contact phase during ground stepping is shown by a signal from a pressure sensor. The trigger signals represent the timing of the TMS. The stimulus intensity was 45% of the maximal stimulator output in this subject. The MEPs evoked by TMS during ground stepping (the area enclosed by the dotted line) are enlarged on the right (from 50 ms before to 100 ms after the stimulation).

Figure 2 illustrates a typical example of the averaged MEPs in all four muscles recorded in the standing posture and under the two stepping conditions in a single subject. The step cycle was divided into six equal phases on the basis of the maximal flexion of the right hip. The top of this figure is a schematic representation of the lower limb with the DGO at the six different phases of the step cycle and the bottom of the figure shows the angle trajectories of the hip and ankle joints under both stepping conditions. Each MEP trace shows an average of six or more responses. During stepping, the MEPs were modulated at phases of the step cycle despite the consistent stimulus intensity. The TA MEPs were especially strongly facilitated during ground stepping. The MEPs in the Sol were larger in the latter part of the stance phase than at the other phases during ground stepping. In the RF and BF, MEPs were also changed across the step cycle. The modulation pattern of the MEPs was obtained in a reproducible fashion across different recording sessions.

While the subject was standing with 40% body weight unloading, the stimulus intensity was chosen in order to evoke MEPs of ~0.1 mV in the TA. The stimulus intensities among the subjects ranged from 41 to 62% of the maximum stimulator output. The average peak-to-peak TA MEP in the upright posture from all subjects was 0.122 ± 0.025 mV. At that intensity, the MEP responses in this posture were detectable in nine of 13 subjects in the RF, six in the BF and 10 in the Sol. The average peak-to-peak MEPs of upright standing were 0.104 ± 0.031 mV in the RF, 0.034 ± 0.007 mV in the BF and 0.091 ± 0.030 mV in the Sol.

Figure 3 shows the mean TMS-evoked MEP responses, BGAs and ankle joint angle during ground and air stepping in all subjects. The mean ankle joint angle during the six step phases remained unchanged in both stepping conditions because of the fixation by the ankle foot orthosis. In the RF, BF and TA muscles, there were no visible BGAs in both stepping conditions. The two-way ANOVA [task (two stepping conditions) × phase (six different phases)] tests showed no significant effects of task, phase and task × phase interaction in the BGAs of each muscle. In the Sol muscle, the small EMG activation was induced at the stance phase of ground stepping but the BGAs in the Sol showed

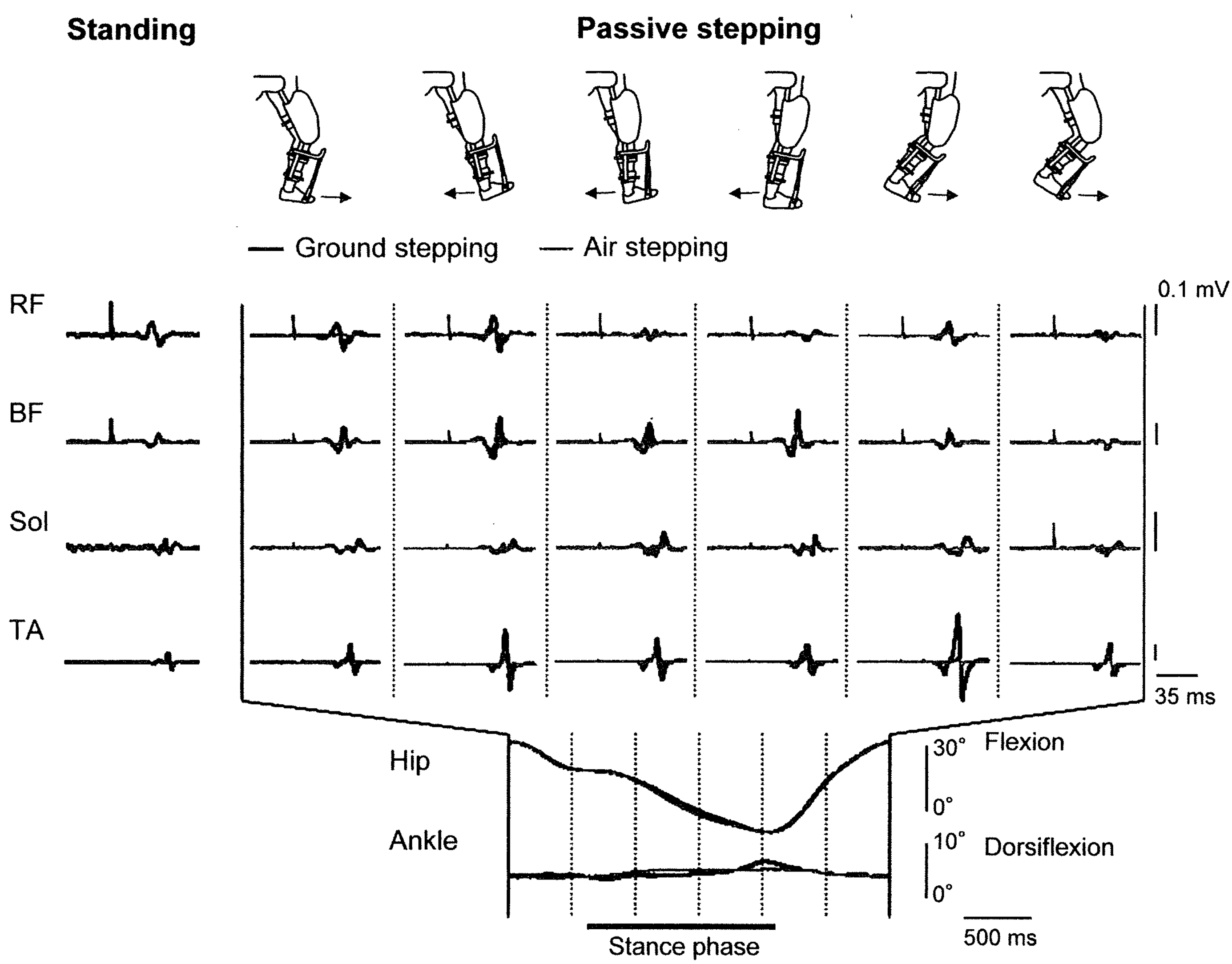

FIG. 2. Typical examples of averaged MEPs in the RF, BF, Sol and TA muscles during upright standing and in each of the six phases during two different passive stepping conditions, and time-courses of the hip and ankle joint angles during stepping in a single subject. The schematic illustrations indicate the right lower limb with the orthosis at six stepping phases. Each MEP trace at each phase is an average of more than six stimuli. The TMS intensity was 41% of the maximal stimulator output.

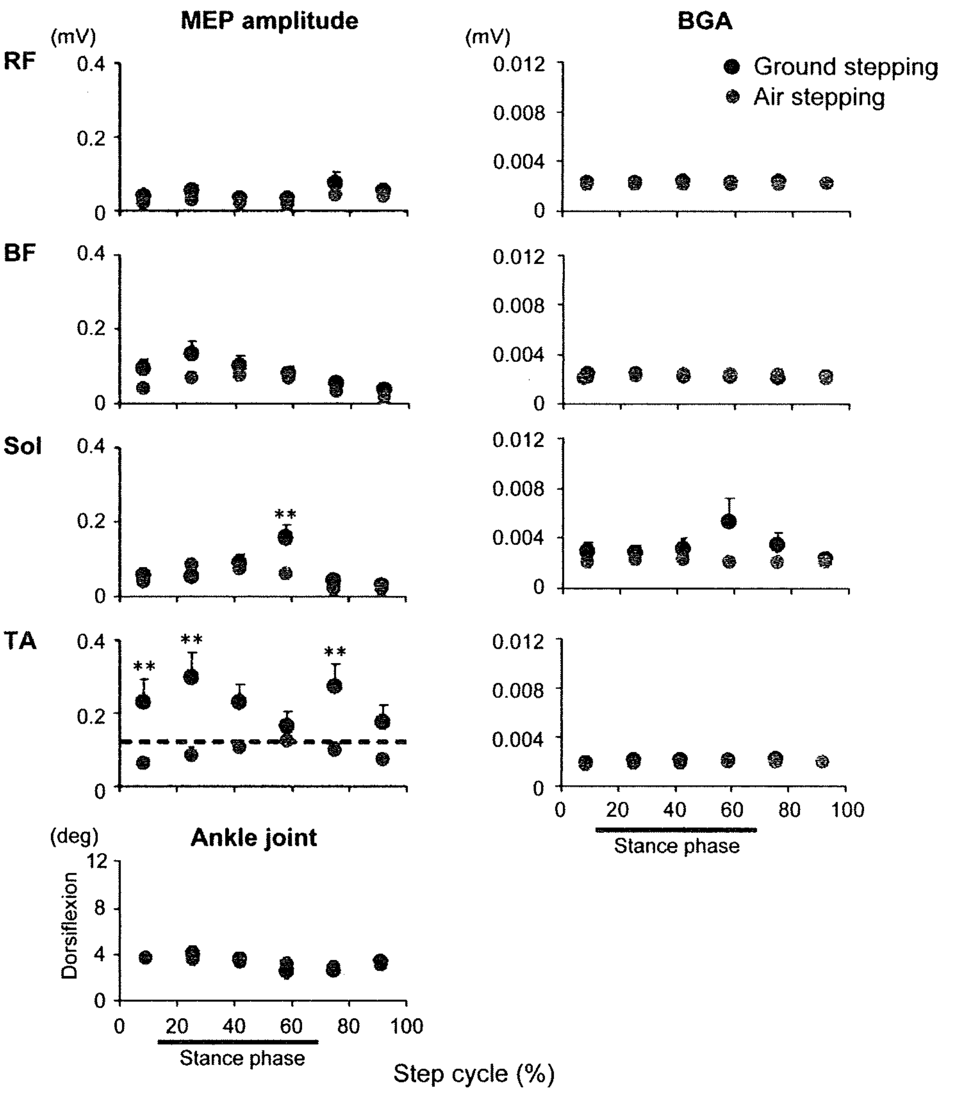

FIG. 3. Averages of the peak amplitude of the TMS-evoked MEPs, EMG BGAs and ankle joint angle recorded at six different phases during ground and air stepping. These values were recorded from four muscles in the right leg. Each plot represents the mean (+SEM) value across all subjects. Significant difference between the values during ground and air stepping (**$P < 0.01$). The dashed line indicates the MEP amplitude in the TA at upright standing with 40% body weight unloading.

no significant effect for task, phase and task × phase interaction. The MEPs in the Sol muscle showed significant effects of task ($F_{1,12} = 9.68$, $P = 0.009$), phase ($F_{5,60} = 7.50$, $P = 0.005$) and task × phase interaction ($F_{5,60} = 4.64$, $P = 0.02$). The one-way ANOVA test indicated that phase difference was significant in the case of ground stepping ($F_{5,60} = 10.39$, $P = 0.002$) but not in the case of air stepping ($F_{5,60} = 2.97$, $P = 0.07$). During ground stepping, the Sol MEP at the late-stance phase was significantly larger than that at the other step phases ($P < 0.05$). There was a significant increase of the MEP amplitude at the late-stance phase during ground stepping compared with air stepping ($P < 0.01$). For the TA MEPs, the ANOVA test indicated that the task difference was significant ($F_{1,12} = 12.59$, $P = 0.004$) but the main effect of phase was not significant ($F_{5,60} = 2.29$, $P = 0.10$). The MEPs in the TA muscle revealed a significant interaction with task × phase ($F_{5,60} = 3.84$, $P = 0.03$), suggesting that the MEP facilitation pattern differed significantly between stepping conditions (Fig. 3). In Fig. 3, the dashed line indicates the MEP response in the TA during upright standing with 40% body weight unloading. The TA MEPs in all phases of ground stepping were larger than at upright standing. The MEPs at the early- and late-swing phases as well as the early-stance phase were significantly different between ground stepping and air stepping ($P < 0.01$). The results of one-way ANOVA for the TA MEPs under each stepping condition revealed that the phase difference was significant in the case of both stepping conditions (ground and air stepping: $F_{5,60} = 2.88$ and 3.88, $P = 0.04$ and 0.01). During ground stepping, the TA MEPs at the early-stance phase were significantly larger than those at the late-stance and mid-swing phases ($P < 0.05$). However, during air stepping, the TA MEPs were significantly larger at the mid- and late-stance phases compared with the late-swing phase ($P < 0.05$). The BF MEPs during passive stepping, except in the mid-swing phase, were larger than at upright standing. The increase of the BF MEPs was observed particularly around the early-stance phase. Although the RF MEPs tended to increase at the swing and early-stance phases in both stepping conditions, the average MEPs in each phase in both stepping conditions were smaller than those during upright standing.

Modulation pattern of the transcranial magnetic stimulation- and transcranial electrical stimulation-evoked responses during passive stepping

As an additional experiment, we compared the modulation patterns of TMS- and TES-evoked MEPs during both stepping conditions in four subjects with a protocol similar to that of the main experiment. As shown in Fig. 4A, the electrically induced MEPs had a latency that was 1.5–2.0 ms shorter than the magnetically induced MEPs in the condition where the MEP amplitudes by TMS and TES were similar. Each MEP response during ground stepping and the mean values of the MEPs at six phases of ground stepping for the same subject as in Fig. 4A are shown in Fig. 4B and C, respectively. Although RF MEPs were not evoked by TMS in this subject, the modulation patterns of TES-evoked MEPs were similar to the TMS-evoked MEPs in other muscles (Fig. 4B and C). In the TA, the MEP facilitation was observed at the early-swing phase and at the swing-stance transition, although differences in TMS- and TES-evoked

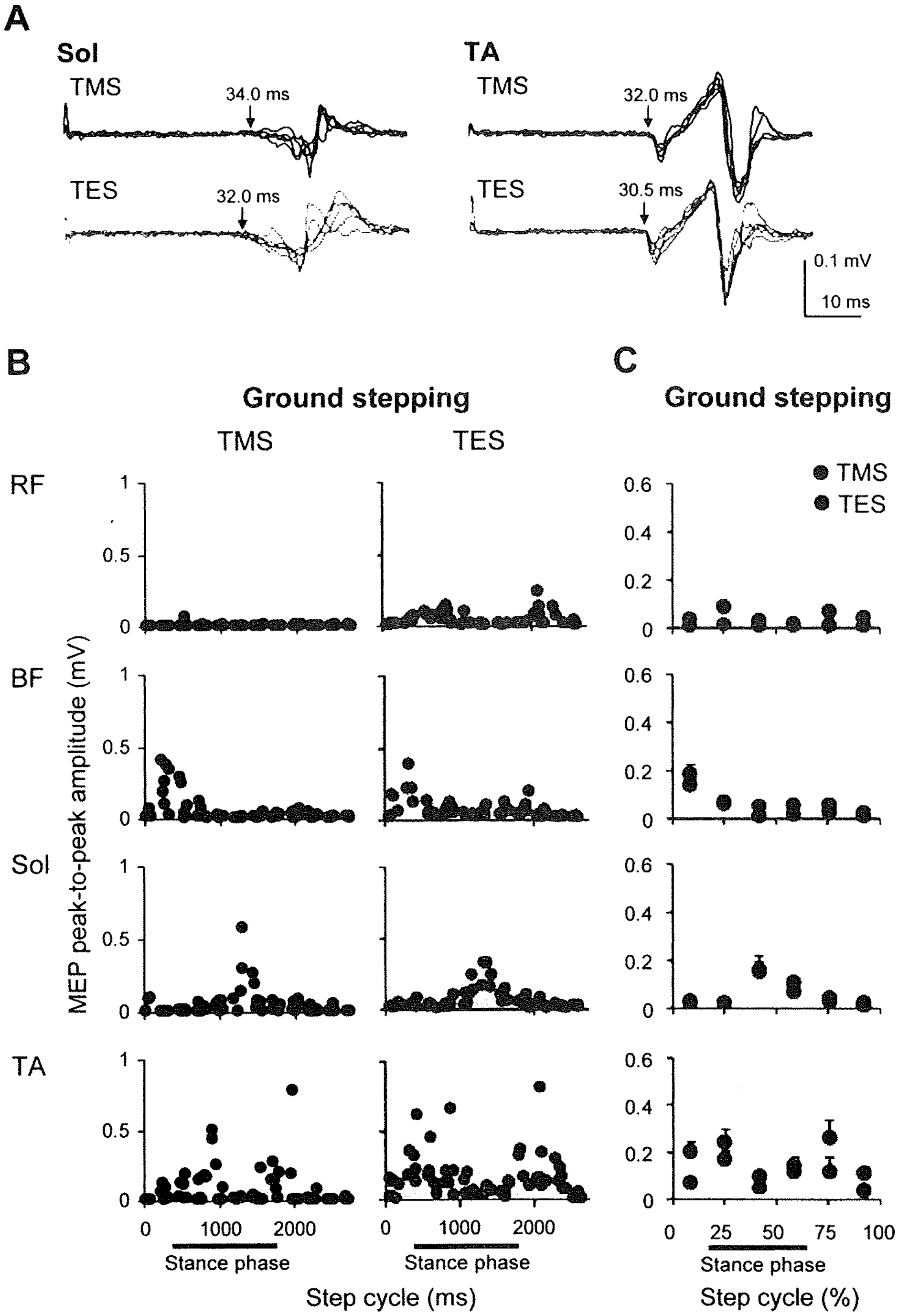

FIG. 4. (A) Raw waveforms of the MEPs evoked by TMS and TES in a subject. Five traces of MEPs are superimposed from the instant of stimulation to 60 ms after stimulation. Latencies evoked by TMS and TES are shown by the arrows. (B) MEP amplitude of the RF, BF, Sol and TA muscles evoked by TMS and TES during ground stepping in the same subject as in A. (C) Averages of TMS- and TES-evoked MEP amplitudes in the RF, BF, Sol and TA muscles at six phases of ground stepping in the same subject. Each plot represents mean (+SEM) value.