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厚生労働科学研究費補助金
障害者対策総合研究事業（身体・知的等障害分野）

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

平成21年度～23年度　総合研究報告書

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総合研究報告書

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

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研究要旨

本研究では、脊髄損傷患者の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・賦活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 (neurorehabilitation) 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影響している。

最近報告された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、その効果に統計学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。

研究方法としては、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した。最終年度には、開発したリハビリプログラムの評価・改善を行った。

（１）不全脊髄損傷患者に対し、適度な荷重・上肢運動の追加・随意的な歩行努力の3要素を加えたプロトコールを用いる週3回のLokomatトレーニングを12週間行い、その効果を前後の測定によって検証した。いずれの被験者においても歩行速度の改善、静止立位姿勢中の重心動揺特性の改善が認められた。

（２）皮質脊髄路興奮性を反映する経頭蓋磁気刺激を用いた運動誘発電位は、6名中3名においてトレーニング当初は発現しなかった前脛骨筋の応答がトレーニング後で発現するなど、中枢神経の可塑的変化を支持する結果が得られた。

（３）下肢筋群全般にわたって痙性麻痺の減少を示唆する変化を認めた。脊髄歩行中枢による歩行のリズム生成は、随意運動出力の改善とあいまって、歩行動作の改善と安定性をもたらしたものと考えられた。

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方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。

研究分担者氏名・所属研究機関名及び所属研究機関における職名

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A．研究目的

脊髄損傷者の歩行機能回復をめざす体重免荷によるトレッドミル歩行訓練は「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

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した。
本研究を開始するに当たり、課題を整理した。
（1）脊髄CPGを賦活化するというコンセプトはあるものの、それ以上のメカニズムが明らかでないこともあり、訓練方法（Lokomatの使用方法も含め）にばらつきがある。その背景として、受傷後1年以内の訓練効果には自然回復の影響があり、他の訓練効果と識別しにくいこと、逆に受傷後1年以降であると訓練効果が出にくいことも考えられる。したがって、研究の組み立てとして、以下を定めた。
・CPG賦活化の方法を検討する。
・訓練前後で得られる歩行の変化を詳細に解析する。
・まず受傷後1年以上経過した症例を対象とする。
そこで、
（2）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を考案、3ヶ月の歩行リハビリテーション実施による訓練効果の定量的把握を行った（図1）。
（3）1年目を脊髄CPGの活動賦活のための方法論づくりに関する基礎研究ステージ、2年目をリハビリテーション方法の開発に関する応用研究ステージ、さらに3年目を開発した方法の評価・改良に関する最終研究ステージとして位置づけた。
（4）応用研究ステージにあたる2年目には、1年目に検討した、脊髄CPGの特性についての知見を応用し、歩行運動出力を適切に促すための具体的な方法の検索と、考案された方法を実際に脊髄不全損傷者に実施することで、歩行機能がどのように変化するかを実験的に検討した。
（5）合わせて脊髄における組織破壊に由来する微量物質（バイオマーカー）を指標に組織損傷の程度を推測し、臨床的機能予後との関連を検討した。
そして
（6）最終研究ステージにあたる3年目（平成23年度）にはまず、2年目終盤に試験的に実施した脊髄損傷者の訓練実験のデータを解析し、訓練プログラムに改善の必要性があるか否かについて検討した。
（7）上肢の随意運動が可能な例では、四肢の連関運動を取り込むこととし、自身の運動努力への集中を高める手法とした。その後、7名の脊髄不全損傷者に対して引き続き3か月にわたる歩行訓練を実施した。

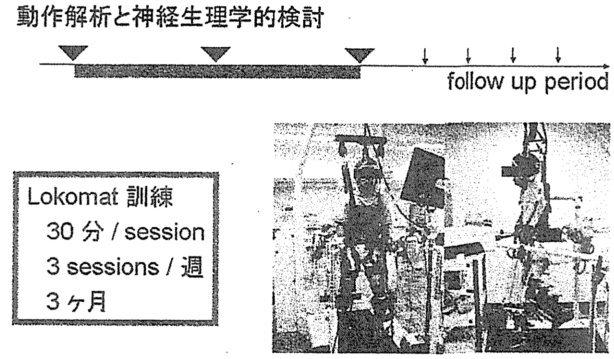


図1 Lokomatを用いた歩行訓練の実際

訓練効果の評価にあたっては、外的な歩容の変化のみならず、神経活動の変化を生理学的にとらえ、歩行機能の改善が中枢神経系のどの階層で、またどのような機序で生じたのかを検討することとした。

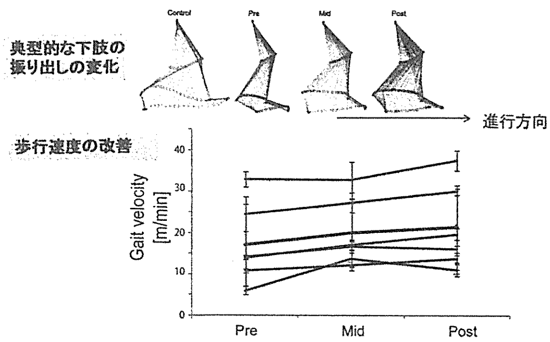
また、訓練終了3か月後の計測を実施することで、訓練終了後の効果の残存についても検討した。

（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した。
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。

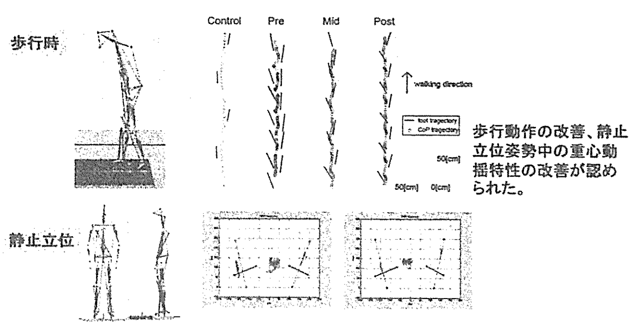
C．研究結果

初年度は、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。
2年度および最終年度は初年度の結果を踏まえ、Lokomatを用いた歩行訓練を施行する際に、適度な荷重・上肢運動の追加・随意的な歩行努力の3要素を加えたプロトコールを作成した。実際に不全脊髄損傷患者に対し、週3回のLokomatトレーニングを12週間行い、その効果を前後の測定によって検証することとした。

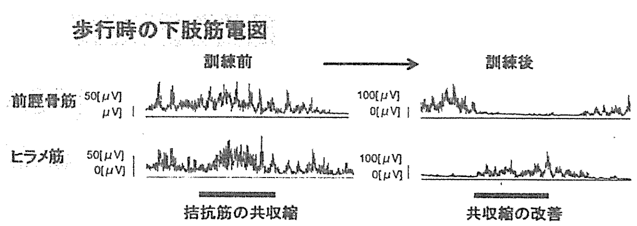
歩行速度の改善



重心の安定性



筋電図の変化



下肢筋の過剰の興奮（痙性を含む）が減弱した結果、筋活動が整うようになった

いずれの被験者においても歩行速度の改善、静止立位姿勢中の重心動揺特性の改善が認められた。皮質脊髄路興奮性を反映する運動誘発電位は、6名中3名においてトレーニング当初は発現しなかった前脛骨筋の応答がトレーニング後で発現するなど、中枢神経の可塑的変化を支持する結果が得られた。また、歩行中の下肢筋活動に関して、体重支持・抗重力的に働く大腿直筋の活動がトレーニング経過に伴って増加する傾向、さらに伸張反射感受性の過剰亢進によって生じると思われる遊脚期後半の一過性の大腿二頭筋の活動がトレーニング後に減弱する傾向を認めるとともに、下肢筋群全般にわたって痙性麻痺の減少を示唆する変化を認めた。

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。

上記の歩行機能回復を示す実験結果は、① 動力歩行装具による受動歩行中の繰り返しの求心性感覚情報が脊髄歩行中枢の活動を喚起したことに起因するもの、② 脊髄歩行中枢による歩行のリズム生成は、歩行運動を構成する基本的要素であり、随意運動出力の改善とあいまって、歩行動作の改善と安定性をもたらしたもの、と考えられた。

Ｅ．結論

１．評価（研究成果）

１）達成度について

３か月のトレーニング実験を研究期間終了までに１０名終了する予定であったが、東日本大震災により一時期実験中断を余儀なくされ、２３年度までに、７名の実験完了となった。

２）研究成果の学術的意義について

本研究の結果は、脊髄歩行中枢の活性化を基盤とした歩行再獲得の神経機序についての仮説を支持する重要な根拠となり、運動機能障害者の歩行機能回復のための効果的な歩行リハビリテーションプログラムを立案するための貴重な知見となるものと考える。

３）研究成果の行政的意義について

本研究では動力歩行装置を用いた歩行訓練を用いたが、得られた知見は、免荷装置を用いたトレッドミル歩行、各種歩行リハビリ機器での歩行リハビリにも十分に汎化可能と思われる。

２．結論

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具体的方法についての知見が得られた。今後は、本研究の成果を発展させ、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を検討していく必要がある。

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G．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

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
		中澤　公孝	歩行のニューロリハビリテーション―歩行の再獲得をめざした理論と臨床	杏林書院	東京	2010	167
Ogata T, Kawashima N, Nakazawa K, Akai M	Molecular and electrophysiological approaches for functional recovery in patients with injured spinal cord	Kansaku K, Cohen LG (ed)	Systems neuroscience and rehabilitation.	Springer	Tokyo	2010	69-78

雑誌

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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
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, Fujita M, Takahashi M, Ogawa 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
Abe MO, Masani K, Nozaki D, Akai M, Nakazawa K.	Temporal correlations in center of body mass fluctuations during standing and walking	Human Movement Science	29	556-566	2010

Sayenko DG, Alekhina MI, Masani K, Vette AH, Obata H, Popovic MR, Nakazawa K.	Positive effect of balance training with visual feedback on standing balance abilities in people with incomplete spinal cord injury	Spinal Cord	48	886-893	2010
Ueno T, Ohori Y, Ito J, Hoshikawa S, Yamamoto S, Nakamura K, Tanaka S, Akai M, Tobimatsu Y, Ogata T	Hyperphosphorylated neurofilament NF-H as a biomarker of the efficacy of minocycline therapy for spinal cord injury	Spinal Cord	49	333-336	2011
Nakajima T, Kitamura T, Kamibayashi K, Komiyama T, Zehr EP, Hundza SR, Nakazawa K	Robotic-assisted stepping modulates monosynaptic reflexes in forearm muscles in the human	Journal of Neurophysiology	106	1679-1687	2011
赤居正美	物性・構造の”Manufacturing” ー人体の組織改編を人為的に起こす(第48回日本リハビリテーション医学会学術集会会長講演)	Japanese Journal of Rehabilitation Medicine	49(2)	63-66	2012
Hayakawa K, Okazaki R, Ishii K, Ueno T, Izawa N, Tanaka Y, Toyooka S, Matsuoka N, Morioka K, Ohori Y, Nakamura K, Akai M, Tobimatsu Y, Hamabe Y, Ogata T	Phosphorylated neurofilament subunit NF-H (pNF-H) as a biomarker for evaluating severity of spinal cord injury patients, a pilot study.	Spinal Cord			e-pub

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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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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.

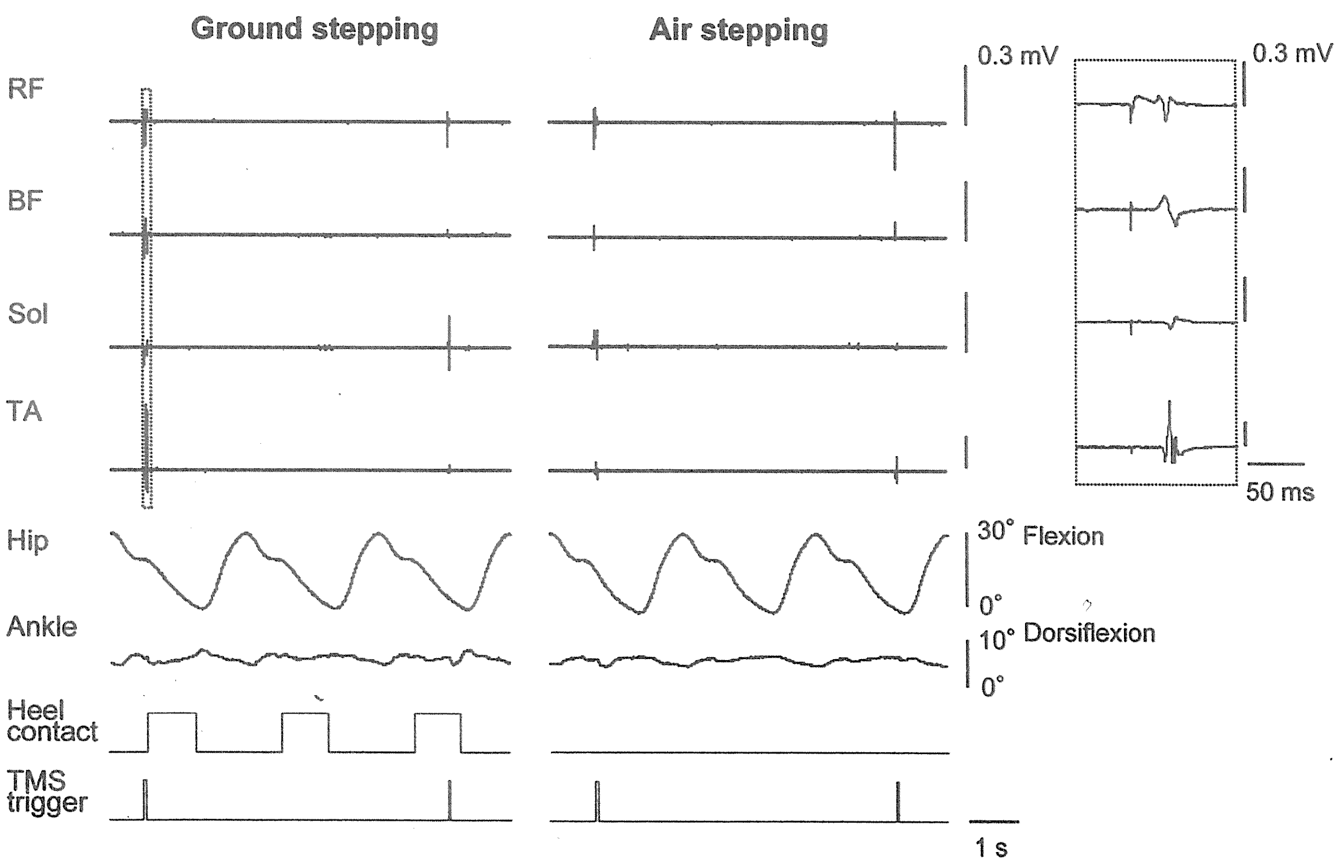

FIG. 1. Example of the raw EMG activities of the RF, BF, Sol and TA muscles and movement trajectories of the hip and ankle joints observed in the right leg during ground and air stepping. The heel contact phase during ground stepping is shown by a signal from a pressure sensor. The trigger signals represent 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

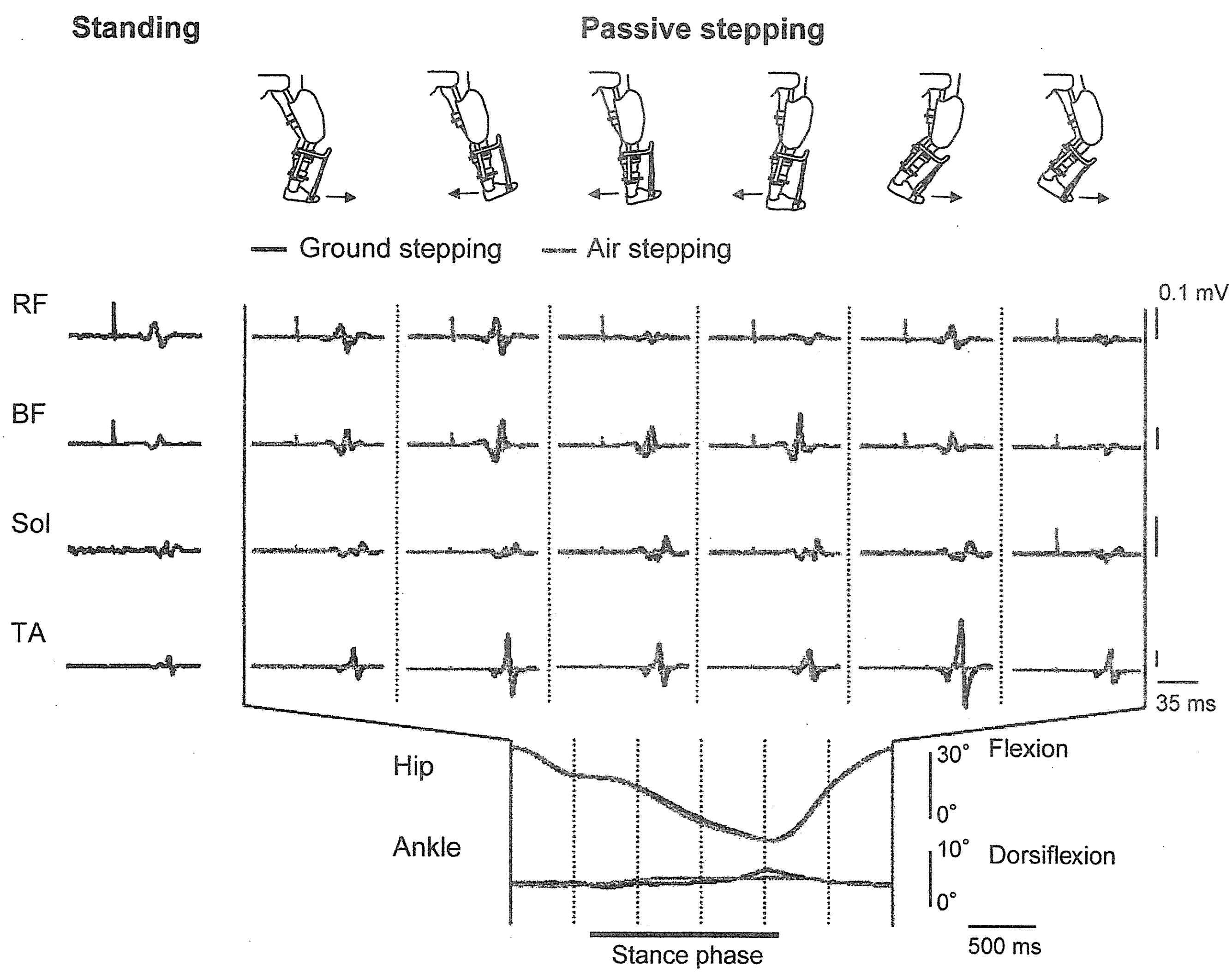

FIG. 2. Typical examples of averaged MEPs in the RF, BF, Sol and TA muscles during upright standing and in each of the six phases during two different passive stepping conditions, and time-courses of the hip and ankle joint angles during stepping in a single subject. The schematic illustrations indicate 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.

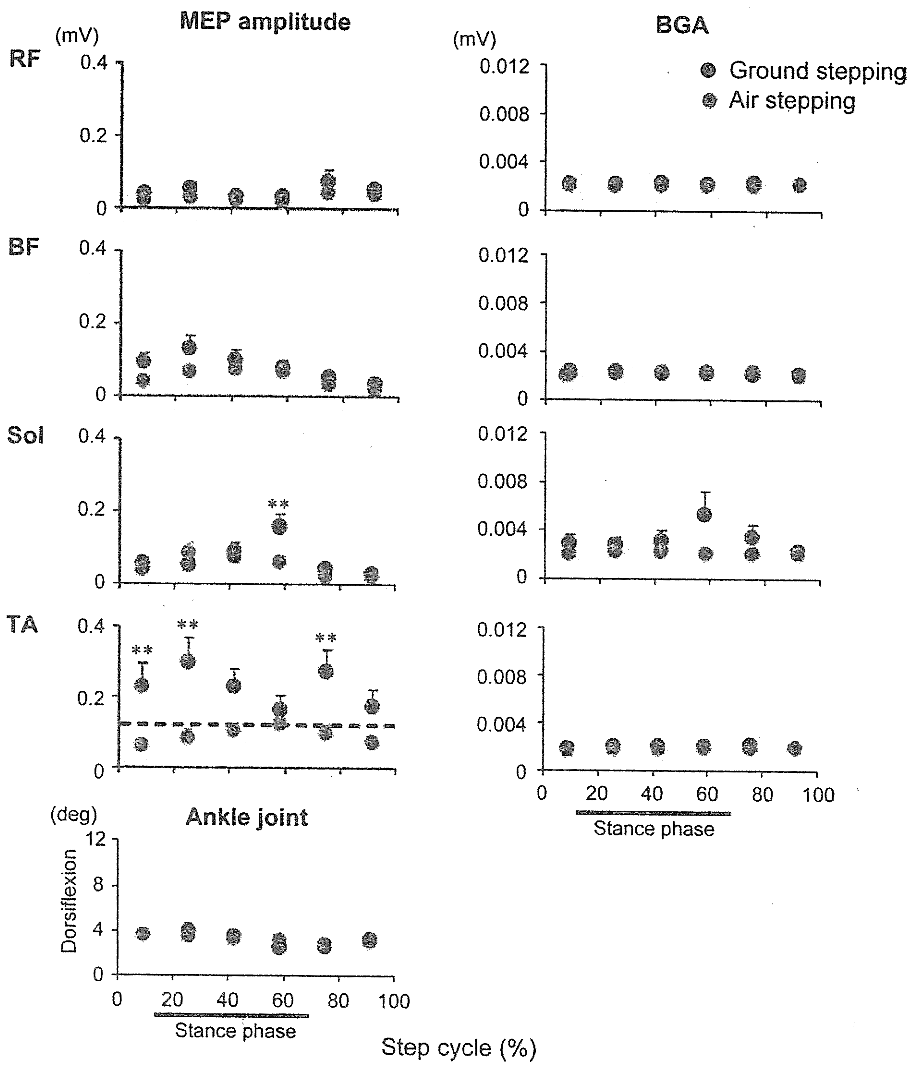

FIG. 3. Averages of the peak amplitude of the TMS-evoked MEPs, EMG BGAs and ankle joint angle recorded at six different phases during ground and air stepping. These values were recorded from four muscles in the right leg. Each plot represents the mean (+SEM) value across all subjects. Significant difference 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

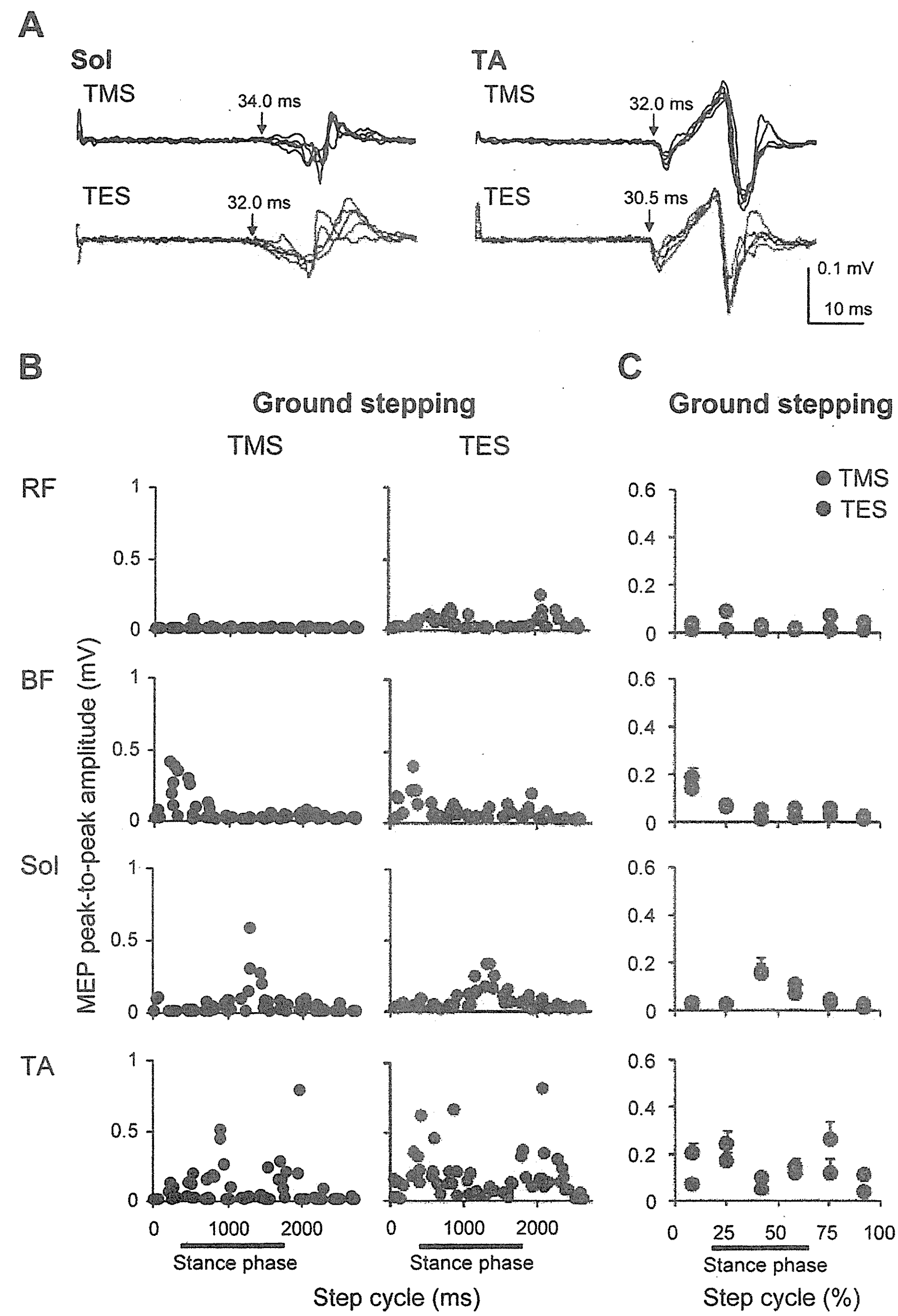

FIG. 4. (A) Raw waveforms of the MEPs evoked by TMS and TES in a subject. Five traces of MEPs are superimposed from the instant of stimulation to 60 ms after stimulation. Latencies evoked by TMS and TES are shown by the arrows. (B) MEP amplitude of the RF, BF, Sol and TA muscles evoked by TMS and TES 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.

MEP sizes were observed (Fig. 4B and C). The TA MEPs in response to TES during air stepping were not facilitated compared with those during ground stepping.

Figure 5 shows the mean TMS- and TES-evoked MEP amplitudes and BGAs in the Sol and TA muscles from four subjects. The stimulus intensity was set to evoke the MEP amplitude of ~0.1 mV in the TA during upright standing with 40% body weight unloading at both stimulus conditions. The mean TA MEP amplitudes during upright standing were 0.120 ± 0.024 mV for TMS and 0.128 ± 0.057 mV for TES in all subjects. The results from all subjects also showed that the MEPs evoked by TES had similar phasic modulation to those evoked by TMS in both stepping conditions.

Discussion

In the present study, TMS was applied to investigate the corticospinal excitability in the lower limb muscles during passive stepping on the ground (ground stepping) and in the air (air stepping) by the DGO. We hypothesized that the corticospinal excitability might be phasically modulated by afferent inputs induced by passive stepping, especially when load-related afferent inputs were included. For this study, the maintenance of the TMS coil position across the step cycle was a crucial point. During stepping, the subjects did not move backwards or forwards due to their fixation to the DGO and harness. Therefore, it appears that, across the step cycle, the coil position for TMS was well stabilized despite the rhythmic lower limb movements during passive stepping. Indeed, we confirmed from video images that the position of the coil relative to the subject's head remained unchanged during the step cycle. The present results showed that the MEPs of the TA muscle during passive stepping were significantly facilitated by load-related afferent inputs.

Motor evoked potential modulation in the tibialis anterior muscle

The DGO could generate stepping motions by motor drives for the hip and knee joint movements. Therefore, the effect of voluntary command was largely reduced during passive stepping by the DGO with the foot lifters. In the present study, no EMG activity in the RF, BF and TA muscles was observed in either stepping condition, whereas Sol EMG activity often occurred at the latter stance phase during ground stepping, which is similar to the findings in a previous study of passive stepping with the same DGO (Dietz *et al.*, 2002; Lünenburger *et al.*, 2006). In the present study, the stepping speed was 1.5 km/h, which was much less than that during normal walking. However, the recording of the EMG activity in the lower limb muscles during treadmill walking over a wide speed range (1–7 km/h) indicated that, although the mean EMG activity increased with speed, a similar pattern of EMG activity was obtained at the different speeds (Ivanenko *et al.*, 2006). In addition, in a previous study that used the

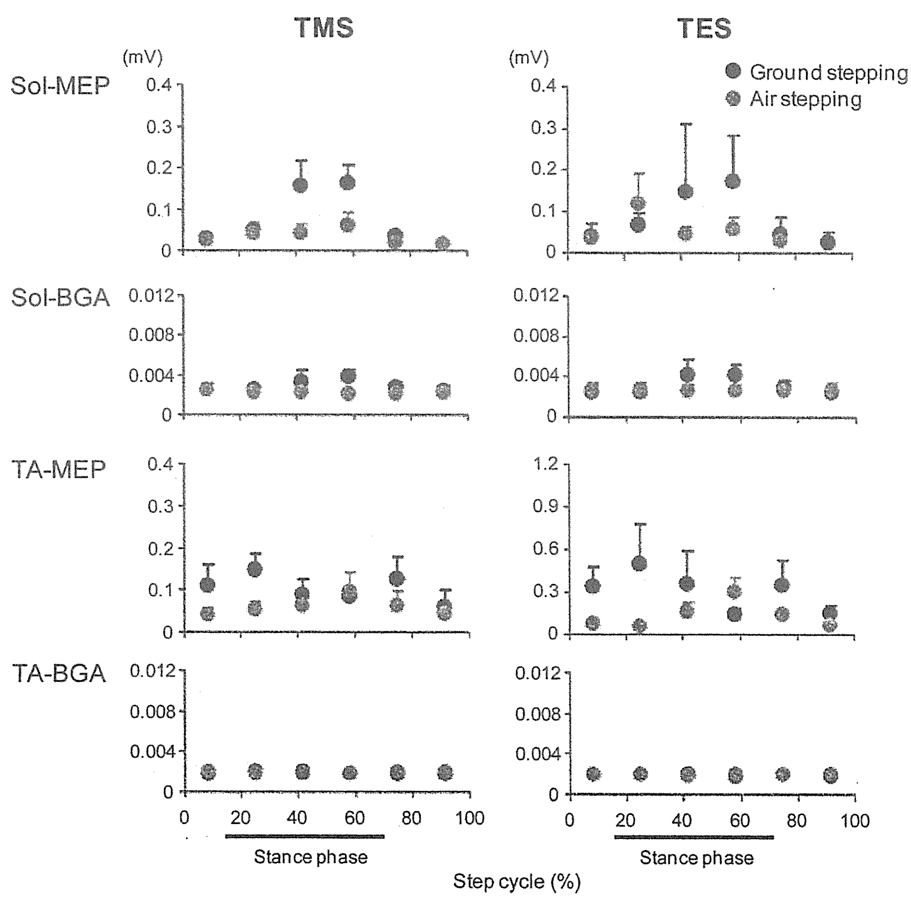

FIG. 5. Averages of TMS- and TES-evoked MEP peak amplitudes and EMG BGAs in the Sol and TA muscles. The black and grey circles depict the average data during ground and air stepping, respectively. Each plot represents mean (+SEM) value across four subjects. The vertical axis of the TA MEP responses induced by TES is three times larger than that of the TA MEP responses induced by TMS.

Lokomat®, locomotor-like EMG activations of the lower limb muscles were observed in complete spinal cord-injured patients during stepping at a speed of 1.5–2.5 km/h (Lünenburger *et al.*, 2006). The result suggests that the spinal neuronal circuits for locomotor activity can be driven by afferent inputs in this speed range in spinal cord-injured patients. In the present study, a noteworthy result was that the TA MEPs evoked by TMS increased at the early- and late-swing phases as well as at the early-stance phase only in the ground stepping condition, whereas no BGAs in the TA were observed during ground and air stepping (Figs 2 and 3). The facilitation pattern of the TA MEPs during ground stepping was similar to that observed during normal treadmill walking, as reported by Schubert *et al.* (1997). These authors found that the TA MEP induced by applying below-threshold TMS was largely modulated in parallel with the TA BGA during treadmill walking.

In the present study, there was phasic MEP modulation in the TA muscle during air stepping (Fig. 3). This result suggests that the corticospinal excitability of the TA muscle is modulated by peripheral afferent inputs associated with locomotor-like movements in the lower limb. However, the TA MEPs during air stepping were similar or smaller than those in the upright standing, whereas MEP facilitation in the TA was observed during ground stepping (Fig. 3). The movement trajectories of the hip and knee joints were not different between ground stepping and air stepping, as the angles were controlled by the computer of the DGO. In addition, the ankle angle was maintained in a constant position with the ankle foot orthosis. Thus, the trajectories of the lower limb joints in both stepping conditions were very similar (Figs 1–3). The factors facilitating corticospinal excitability of the TA muscle during ground stepping appear to be the load-related afferent inputs. The significance of load receptor input for regulating locomotion has been stressed not only for animals but also for humans (Stephens & Yang, 1999; Dietz & Duysens, 2000; Faist *et al.*, 2006; Nakajima *et al.*, 2008). The load-related effects may be mediated by afferent information from the Golgi tendon organs, joint receptors and cutaneous mechanoreceptors (Van de Crommert *et al.*, 1998; Dietz & Duysens, 2000; Faist *et al.*, 2006). By using the same DGO as in the present study, we recently observed facilitation of the cutaneous reflex in the TA muscle by tibial nerve stimulation during passive ground stepping but not during air stepping (Nakajima *et al.*, 2008). The facilitation of the cutaneous reflex was observed at the stance-swing transition during ground stepping (Nakajima *et al.*, 2008), whereas the facilitation of the MEP response in the present study occurred at the early-swing phase and swing-stance transition. Thus, the step phases at which the facilitation effects are mediated were different between the cutaneous reflex and corticospinal pathways. However, the modulation patterns of both the cutaneous reflex and corticospinal pathways during passive-loaded stepping are similar to those observed during normal walking (Yang & Stein, 1990; Schubert *et al.*, 1997). From animal studies, it is well known that spinal neuronal circuits can be activated passively by adequate afferent inputs that might be induced during normal locomotion (Grillner, 1981; Rossignol *et al.*, 2006). Such activities of the neuronal circuits induced during ground stepping may rhythmically facilitate corticospinal excitability of the TA muscle.

Although the main purpose of the present study was to investigate the modulation pattern in corticospinal excitability during passive stepping and not to clarify the level of neuroaxis at which the facilitation is mediated, TES was applied under the same stepping conditions as an additional experiment. As a method of obtaining information about the level (cortical or subcortical) at which a given change occurs, comparison of MEPs evoked by TMS and TES has been proposed (Petersen *et al.*, 2003). With the appropriate stimulation-electrode configuration, it is generally accepted that the current induced by TES at intensities just above the MEP threshold mainly activates the axons of corticospinal cells directly, whereas TMS activates the corticospinal cells either directly close to the cell soma or indirectly through the activation of fibers or neurons projecting onto corticospinal cell (Day *et al.*, 1989; Nielsen *et al.*, 1995; Rothwell, 1997; Di Lazzaro *et al.*, 2001). Therefore, it is considered that the MEPs induced by TES are not influenced by the change in excitability at the cortical levels. The latency of TES-evoked MEPs occurred 1–2 ms earlier than that evoked by TMS in the present study (Fig. 4A). This probably reflects the fact that TES activates the axons of corticospinal cells deep in the cortex (Nielsen *et al.*, 1995). If TES-evoked MEPs remained unchanged between ground and air stepping, whereas TMS-evoked MEPs during ground stepping were larger than those observed during air stepping, it can be concluded that the change of the corticospinal excitability probably occurred within the cortical level. The results of the TA MEPs by TES during ground stepping, however, also showed similar modulation to those by TMS (Figs 4B and C, and 5). Therefore, the level of neuroaxis at which the MEP facilitation in the TA muscle occurred during ground stepping could not be determined from the present study. As the MEP responses by TMS were measured without TA muscle activity, we could not determine the change in the subliminal fringe in the α-motoneurons during ground stepping. In addition, the change of the excitability in the segmental interneurons to the MEP facilitation was not eliminated. Further experiments are needed to specify the neuroaxis underlying facilitation of corticospinal excitability.

Motor evoked potential modulation in other lower limb muscles

The MEP modulation during ground stepping appeared not only in the TA but also in the Sol muscle. In the Sol muscle, weak EMG activity was observed at the late-stance phase during ground stepping and MEPs at this phase increased in a similar fashion (Figs 2 and 3). The EMG activity appeared to be reflexively generated because the stepping was performed passively (i.e. largely reduced the voluntary command). It has been indicated that the load receptors contribute to the afferent-mediated enhancement of ankle extensor muscle activity at the late stance of walking (Grey *et al.*, 2007). As simple rhythmic muscle stretching or loading alone does not lead to a locomotor EMG pattern (Dietz *et al.*, 2002), a combination of locomotor-like afferent inputs would be necessary to evoke locomotor EMG activities. In accordance with the previous study, the Sol EMG activity during air stepping was not observed in the present study. The phase-dependent Sol MEP modulation induced by TES during ground stepping was similar to that induced by TMS (Figs 4B and C, and 5). Therefore, the MEP facilitation would be mainly attributable to changes in the increased excitability of the α-motoneurons in this muscle. In the case of the locomotor activity, the flexors are considered to be predominantly under central control, whereas the extensors are mainly activated by afferent feedback (Dietz, 2002). Therefore, it is interesting that the TA MEP was facilitated by the load-related afferent feedback during passive stepping.

The MEP amplitudes in the upper leg muscles were also changed depending on the step phase (Figs 2 and 3). In the RF, MEPs increased from the initial swing to the swing-stance transition phase during stepping. With regard to the BF, MEPs showed larger amplitudes around the early-stance phase. These phases of MEP facilitation corresponded to the phases at which EMG activities in the respective muscles were seen during normal walking (Winter & Yack, 1987) and active stepping with the DGO (Lünenburger *et al.*, 2006). Bonnard *et al.* (2002) observed stimulation-induced hip movements by above-threshold TMS to the motor cortex of the leg area during treadmill walking. When TMS was applied at the initial swing phase, the stimulation increased the movement of the hip flexion, whereas TMS applied at the mid-stance phase increased hip extension. These results seem to suggest that the excitability of the corticospinal tract in the RF was relatively increased at the initial swing and that the excitability to the BF was relatively increased at the mid-stance. In the present study, similar MEP modulation patterns were induced during passive stepping. As the stimulus intensity was weak for the RF and BF MEPs, the effects of the task and/or step phase on the MEPs may be shown more clearly by investigating the input (stimulus intensity)–output (MEP amplitude) curve of the corticospinal tract at pre-determined step phases (Devanne *et al.*, 1997).

Clinical implications for rehabilitation

In a study using TMS, Thomas & Gorassini (2005) found that intensive locomotor training for patients with incomplete spinal cord injury increased the corticospinal excitability in the lower limb muscles in association with the recovery of locomotor ability. The present data show that the corticospinal excitability to the TA muscle is facilitated during passive ground stepping but not during passive air stepping. This indicates that load-related afferent inputs have a role in amplifying the corticospinal excitability in the TA muscle during stepping. In the locomotor training of patients with spinal cord injury, it is already known that body reloading is crucial to the generation of locomotor EMG activity and recovery of locomotion (Harkema *et al.*, 1997; Dietz & Harkema, 2004; Nakazawa *et al.*, 2004). On the basis of the present results, we cannot determine the effect of passive DGO stepping on the functional recovery of locomotion ability in patients with incomplete spinal cord injuries. However, the supply of appropriate load-related afferent inputs during locomotor training by passive stepping may partly be useful for activating the neuronal circuitry for the locomotion because the MEP responses during passive stepping were facilitated – a similar facilitation pattern to that seen in normal walking. Knowledge about the neuronal control of human locomotion, including passive stepping, should be accumulated to identify a more effective approach for locomotor recovery.

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Abbreviations

BF, biceps femoris; BGA, background activity; DGO, driven-gait orthosis; EMG, electromyographic; MEP, motor evoked potential; RF, rectus femoris; Sol, soleus; TA, tibialis anterior; TES, transcranial electrical stimulation; TMS, transcranial magnetic stimulation.

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