scheduled session, the participant was asked to come to a replacement session. Each session lasted up to 60 min with a total standing time of at least 30 min.

Training protocols

In the 'circle' exercise (Figure 1a), a target moved around the center of the screen. The participant was instructed to track the target and hold the COP indicator over it. In the 'target' exercise (Figure 1b), the participant was asked to keep the COP indicator in the center of a target as still as possible. In the 'hunting' exercise (Figure 1c), a target appeared on the screen in random locations. Once the COP indicator was held 'still' within the boundaries of the target for 3 s, the target would reappear in a different location. In the 'octahedron' exercise (Figure 1d), eight targets were presented at 45-degree angles from one another around the center. The participant was asked to move the COP indicator to each target, and hold the position for 5 s. In the 'basketball' exercise (Figure 1e), three targets (balls) of different color appeared on the top of the screen. The participant was asked to 'capture' the target, and 'drag' it into the basket of the matching color. In the 'ski' exercise

(Figure 1f), the participant was asked to simulate downhill skiing.

The duration of each exercise varied from 1 to 2 min. The score was calculated based on the accumulated time that the COP indicator was over the targets or/and based on the number of successful trials. The exercises were presented in random order. Once a consistent score in each exercise was attained by the participants, the difficulty level of the exercise was increased. The initial difficulty of the exercises was adjusted to each participant based on their performance during the familiarization session. During each training session, an equal number of rounds of each exercise was presented to the participant.

Exercise performance

During the training period, the level of the performance and the rate of learning were monitored. The performance for each exercise was expressed as a percentage of the initial score on the first session. A one-way ANOVA with repeated measures ($\alpha = 0.05$) and a subsequent Dunnet's test were applied to the pooled data. We estimated the rate of learning for different exercises by performing a regression analysis
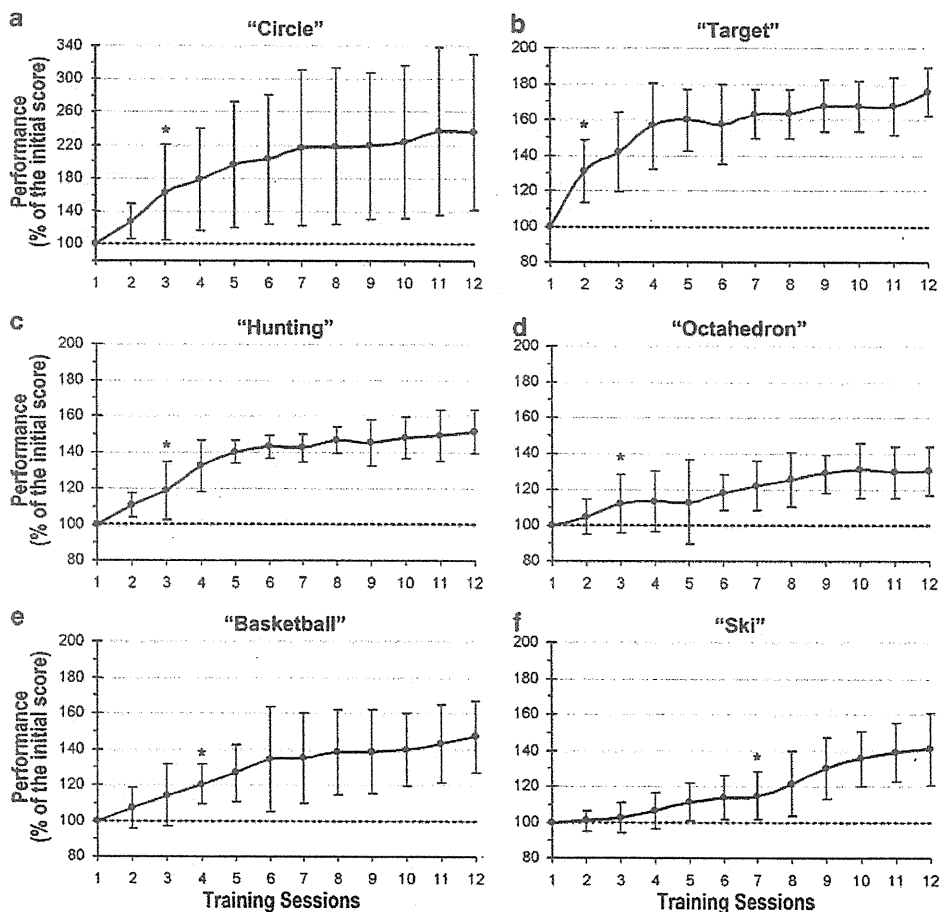

Figure 2 Pooled data showing the performance throughout the training period for all six exercises: (a) 'circle,' (b) 'target,' (c) 'hunting,' (d) 'octahedron,' (e) 'basketball,' and (f) 'ski.' Shown are the percentages of the initial score values obtained in the first session of the training (mean ± s.d.). Asterisks indicate the first session of the training for which the performance was significantly different from the performance during the initial session of the training ($P < 0.05$).

using a logarithmic model in which the rate of learning was proportional to the logarithm of the learning time. The model was described using the following equation:[7]

$$y = a + b \times \log_{10} d,\qquad(1)$$

where y is the expected performance on the day of the training d, and a and b are the regression coefficients, describing the initial level of performance and slope, respectively. To compare the rate of learning across exercises, confidence intervals (CIs) of the slopes were computed using the following equation:

$$\mathrm{CI} = S \pm Z_{\alpha/2} \times (\text{s.e.}/n^{1/2}),\qquad(2)$$

where S is a value of the slope, α is a significance level, Z is a z-score for a two-tailed distribution equal to 1.96, s.e. is the standard error of the measure, and n is the number of the training session. The desired width of the CI was 95%.

Postural stability assessment

Before and after the training period, two different aspects of balance were evaluated: *static* and *dynamic stability*.

During the static stability test, the participant was instructed to stand on the force plate as still as possible for 60 s with the eyes open. After 2 min of rest, the task was repeated with the eyes closed. The fluctuations of the COP were analyzed with root mean square distance (RDIST), the 95% confidence ellipse area (AREA-CE), and the mean velocity (MVELO).[21–23]

During the dynamic stability test, the ability to voluntarily displace the COP to a maximum distance without losing balance was assessed.[24] Eight targets were presented on the screen at 45-degree angles from one another around the center. The participant was instructed to shift the COP indicator as far as possible toward a target, which changed its color, hold this position, and then return the COP indicator to the center. The target was active for 7 s. The average amplitudes of the COP displacements were defined for each direction for the time interval from 3 to 6 s, and were then used as vertices of an octagon. The area of this octagon was defined as the stability zone (AREA-SZ) and was calculated using the following formula:

$$S = \frac{1}{2} \sum_{i=0}^{n-1} (x_i y_{i+1} - x_{i+1} y_i)\qquad(3)$$

where x is the anterior–posterior position of COP and y is the medial–lateral position of COP.

For each measure, comparisons between values before and after the training were performed using a paired t-test. The level of significance was set at $\alpha = 0.05$ for all analyses. The results for the pooled data are presented as mean values and s.d.

Results

Exercise performance

Figure 2 shows the pooled data of the participants' performance throughout the training period in all exercises as a percentage of the initial scores values obtained on the first day of the training. The most prominent performance was revealed in the exercises 'circle' (Figure 2a) and 'target'
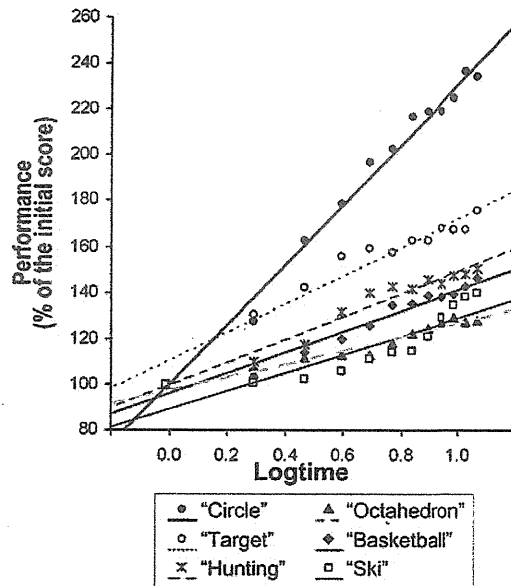

Figure 3 Regression curves representing the pool-average rate of learning for different exercises during the training period (logarithmic model). The abscissa indicates the log10 of the number of training session.

(Figure 2b): at the end of the training period, the average score reached 236 ± 94, and $176 \pm 14\%$ of the initial values, respectively. Somewhat similar yet lower performance was observed in the exercise 'hunting' (Figure 2c): the score on the 12th session reached $151 \pm 12\%$ of the initial value. A lower level of performance improvement occurred in exercises 'octahedron,' 'basketball,' and 'ski' (Figures 2d–f): the score on the 12th training session increased in comparison with the initial values, reaching 130 ± 14, 147 ± 20, and $141 \pm 20\%$, respectively.

The results of the regression analysis (Figure 3) revealed that the most significant changes in the learning rate occurred in the exercises 'circle' and 'target': the slope of the regression curves in the logarithmic model reached 56.9 (CI from 30.3 to 83.5) and 26.9 (CI from 23.0 to 30.8), respectively. Lower learning rates occurred in the exercises 'hunting,' 'basketball' and 'ski,' where the slope of the regression curve reached 21.5 (CI from 17.2 to 25.9), 19.6 (CI from 13.1 to 26.1), and 17.2 (CI from 12.5 to 21.9), respectively. Finally, the slowest learning rate took place in the exercise 'octahedron,' where the slope of the regression curve reached 12.7 (CI from 10.1 to 15.2).

Postural stability

In Figure 4, the pooled data of RDIST (Figure 4a), MVELO (Figure 4b), and AREA-CE (Figure 4c) are depicted for the performance before and after the balance training. The results show that all measures except MVELO of the medial–lateral COP fluctuation were significantly decreased after the balance training (Table 2).

During the test of dynamic stability, AREA-SZ was significantly increased after the training period, reaching $221 \pm 86\%$ of the pre-training values (Figure 5; Table 2).
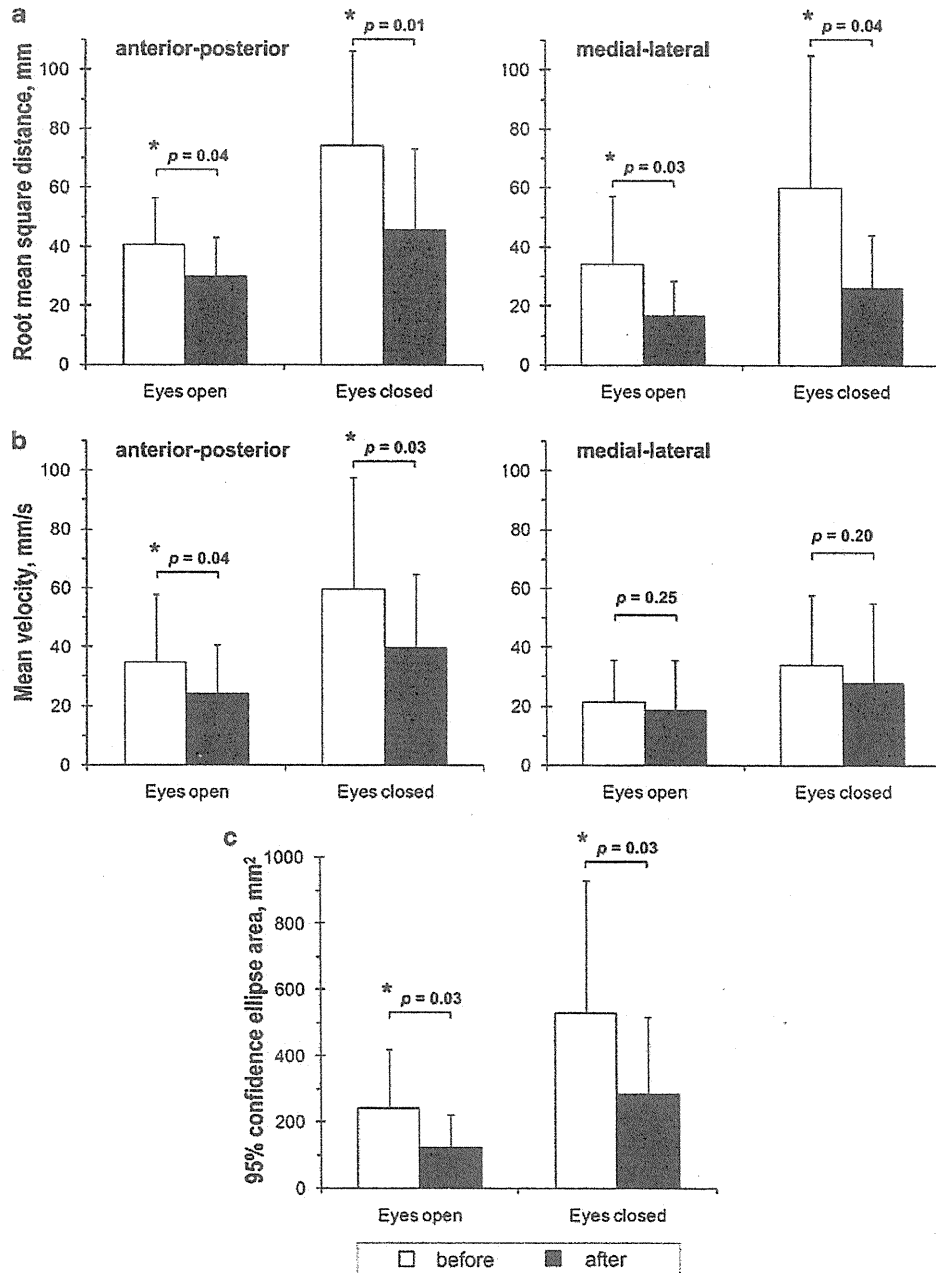
Figure 4 Test of static stability. Pooled data showing (a) the root mean square distance (RDIST), (b) the mean velocity (MVELO), and (c) the 95% confidence ellipse area (AREA-CE) of the COP fluctuation during standing with eyes open and eyes closed before (white columns) and after (black columns) the balance training (mean ± s.d.). RDIST (a) and MVELO (b) during anterior–posterior and medial–lateral fluctuations of the COP are shown on the left and right panels, respectively. Asterisks indicate statistically significant differences between the values before and after the training ($P < 0.05$).

Discussion

In this study, two main results were found. First, after the balance training with visual feedback, all participants showed substantial improvement in the scores of each exercise, though the achieved performance and rate of learning varied across different exercises. Second, the balance performance during both static and dynamic assessment was significantly improved after the training.

Improved balance function

Two types of supervised learning conditions were implemented during the balance training.[7] For the first type ('circle' and 'target'), a given stereotyped pattern of movement had to be generated, requiring a high precision of movement performance. For the second type ('basketball' and 'ski'), the participants apparently applied a general strategy of voluntary postural control that included attention, decision making, and performance of the task with

Table 2 Average values of the RDIST, MVELO, AREA-CE, and AREA-SZ after the balance training (mean ± s.d.)

Measure	Condition	Anterior–posterior (% of initial values)	Medial–lateral (% of initial values)
RDIST	EO	75 ± 17	61 ± 35
	EC	60 ± 25	38 ± 30
MVELO	EO	73 ± 20	85 ± 30
	EC	66 ± 25	79 ± 34
AREA-CE	EO	52 ± 32	
	EC	46 ± 25	
AREA-SZ	EO	221 ± 86	

Abbreviations: AREA-CE, 95% confidence ellipse area; AREA-SZ, area inside the stability zone; MVELO, mean velocity; RDIST, root mean square distance.
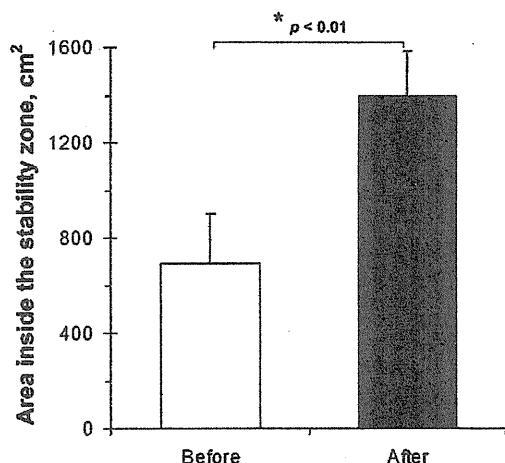

Figure 5 Test of dynamic stability. Average values of the area inside the stability zone before (white column) and after (black column) the balance training (mean ± s.d.). Asterisks indicate statistically significant difference between the value before and after the training ($P < 0.05$).

different movement patterns. In addition, mixed conditions ('hunting' and 'octahedron') were used during the training. Our analysis revealed that the most successful improvement was achieved in exercises of the first type that presented the same movement pattern again and again, whereas less progress was obtained in exercises with different movement patterns. The lowest performance and learning rate during the exercise 'octahedron' might be explained by a greater muscle activity during this exercise; thus, the improvement of muscle performance occurred with a lower increment than enhancement in postural synergies and strategies.

Evidence from human studies has shown that goal-oriented and task-specific training improves impaired function after central and peripheral nervous system disorders or lesions.[14–16] Presumably, an increase in cortical control of muscles after incomplete SCI might allow functional recovery through the development of alternative movement strategies.[25] As a result, the motor programs for balance control strategies, provided by *task-specific* training, seemed to be effective and could affect the final outcome of the participants in our study.

At the same time, both static and dynamic stability tests did not correspond directly to the motor tasks engaged throughout the training period. Nonetheless, both static and dynamic stability tests (including eyes-closed condition during the static test) revealed a significant improvement of postural control after the training period in all participants. It has been earlier shown that during static postural stability test, RDIST and AREA-CE can be related to the effectiveness of, or the stability achieved by, the postural control system; and MVELO has been related to the amount of regulatory activity associated with this level of stability.[21–23] The increased AREA-SZ on the other hand has been related to an enhancement of muscle strength.[24] Consequently, we can also assume a *non-specific* effect of the training on the postural control mechanisms after our balance training program.

Potential mechanisms

The central nervous system of individuals with incomplete SCI is susceptible to substantial reorganization as cortical, subcortical, and much of the local spinal cord circuitry remain largely intact and still partially interconnected by unlesioned fibers.[25] Although any of the adaptive reorganizations might contribute to the exhibited improvement, we turn our attention toward the main function of supraspinal reorganization (plasticity) as the mechanism most likely associated with cognitive processes—namely, the formation of internal models and learning of limits.

It has been suggested in studies with stroke survivors that by giving the participants additional visual information, they became more aware of the body's displacements and orientation in space,[13] were able to integrate somatosensory and visual information in relation to stance and movements,[26] recalibrate deficient proprioceptive information,[13,27] and compensate the sensorimotor deficit.[10] We hypothesize that in individuals with SCI, mechanisms of balance improvement because of altered sensorimotor integration and more extensive processing of residual proprioceptive and cutaneous sensory information also seem feasible.[25,28–30]

Our training program provided a progressive challenge and overload to the postural control system throughout the training period.[31] We assume that such activity *per se* could improve the strength and endurance of muscles participating in control of posture, especially in participants with minimal function before the training.[24,32,33] Furthermore, our balance training program included exercises that closely mimicked reaching in standing tasks, thereby providing muscle activation associated with functional challenge of maintaining balance.[24,34–36] We, therefore, suggest that the improved function during dynamic tasks might be at least partially attributed to enhancements of the muscle properties.

Study limitations and future directions

Further studies in a larger group of individuals with SCI are required to confirm our observations. Ideally, these studies would include a control group and clinical information

(for example lower extremity motor score) as well as measures of activity limitation and participation restriction to determine the clinical impact and functional consequences of balance training with visual feedback. In addition, muscle strength and aerobic capacity have to be measured in important postural muscles.

Conclusion

As the first report in this field, we showed that individuals with chronic incomplete SCI show improvements in upright static and dynamic postural control after balance training with visual feedback during standing. Although our observations have to be confirmed in further studies, we assume that balance training with visual feedback opens up a possibility to supplement routine rehabilitative interventions in individuals with incomplete SCI. The main positive effect of the balance training on postural control may be associated with the improvement of existing and the development of new motor strategies, sensorimotor integration, and a direct effect of the training on the muscles' functional properties.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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ORIGINAL ARTICLE

Hyperphosphorylated neurofilament NF-H as a biomarker of the efficacy of minocycline therapy for spinal cord injury

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Study design: An *in vivo* study in a rat model of acute spinal cord contusion.

Objectives: To assess the efficacy of novel therapies for acute spinal cord injury (SCI), methods to evaluate accurately the effects of these therapies should be developed. Although neurological examination is commonly used for this purpose, unstable clinical conditions and the spontaneous recovery of neurological function in the acute and subacute phases after injury make this measurement unreliable. Recent studies have reported that the phosphorylated form of the high-molecular-weight neurofilament subunit NF-H (pNF-H), a new biomarker for axonal degeneration, can be measured in serum samples in experimental SCI animals. Therefore, we aimed to investigate the use of plasma pNF-H as an indicator of the efficacy of minocycline, a neuroprotective drug, for treating SCI.

Setting: This study was carried out at Saitama, Japan.

Methods: Spinal cord injured rats received either minocycline or saline intraperitoneally. The plasma pNF-H levels and functional hind limb score were determined after the injury.

Results: Minocycline treatment reduced plasma pNF-H levels at 3 and 4 days post-injury (dpi). Rats with lower plasma pNF-H levels at 3 dpi had higher hind limb motor score at 28 dpi.

Conclusions: pNF-H levels may serve as a biomarker for evaluating the efficacy of therapies for SCI.

Spinal Cord advance online publication, 31 August 2010; doi:10.1038/sc.2010.116

Keywords: biomarker; pNF-H; minocycline; spinal cord injury

Introduction

The sensorimotor dysfunction that occurs after spinal cord injury (SCI) results from both the primary mechanical insult and secondary damage, which includes multiple components such as inflammatory reaction, delayed neuronal and glial cell death, and axonal degeneration.[1,2] These biological processes continue for several days, and can therefore be targeted in therapeutic intervention. Various therapeutic strategies have been developed to ameliorate tissue loss arising due to secondary damage.[3]

Appropriate assessment methods are required to determine the efficacy of novel neuroprotective therapies. The American Spinal Injury Association assessment scale is a standardized tool that is used widely to assess neurological state (grade A–E) and motor score in SCI patients. However, the spontaneous neurological recovery in acute and subacute SCI patients may limit the reliability of assessments of the initial state.[4] Further, as the degree of spontaneous recovery varies among patients in clinical trials of SCI therapies, it is unclear whether the neurological improvement is because of the therapeutic intervention or spontaneous recovery. Therefore, a novel technique that is independent of neurological status is required for monitoring progressive tissue damage; this development will facilitate further clinical trials of SCI therapies.

The severity or stage of a disease is usually determined by measuring the levels of certain biomarkers in blood or cerebrospinal fluid. In the clinical field of traumatic brain injury, several proteins that are synthesized in neurons and glial cells, such as S100B, neuron-specific enolase, myelin basic protein and glial fibrillary acid protein, have been proposed as surrogate markers that can be used in clinical trials.[5,6] However, there are only a few reports on biomarkers in SCI patients.[7,8]

Recently, studies in experimental SCI animals and aneurismal subarachnoid hemorrhage patients revealed an association between the level of the phosphorylated form of the high-molecular-weight neurofilament subunit NF-H (pNF-H) in blood and prognosis.[9,10] Because NF-H is one of the

major structural complexes in axons, increased levels of the phosphorylated form of the protein, which is resistant to proteases, indicate increased axonal damage. In contrast to the other neural-cell-derived biomarkers such as serum S100B and neuron-specific enolase, whose levels peak within 24 h after injury, serum pNF-H levels peak 3 days after SCI.[9,11] This temporal pattern may reflect progressive axonal loss due to secondary damage. A previous study in experimental SCI rats reported a relation between the serum pNF-H level and the intensity of the initial impact (primary insults) delivered by the SCI device.[9] However, whether pNF-H can be used as a marker to evaluate the efficacy of therapeutic interventions against secondary damage in SCI remains unknown. In this study, we measured the plasma pNF-H levels in SCI rats treated with minocycline, a neuroprotective drug.[3,12,13]

Materials and methods

Surgical procedures

All surgical procedures were approved by the ethical committee of Research Institute, National Rehabilitation Center. Experimental SCIs were induced in adult 10- to 12-week-old Sprague–Dawley rats (body weight, 280–320 g). Animals were anesthetized by intraperitoneal injection of barbiturate. Thereafter, the lower thoracic lamina was exposed, and the lamina was removed at the level of Th10. Contusion injuries were then induced by using the Infinite Horizon SCI device (Precision Systems and Instrumentation, Lexington, KY, USA). The intensity of the device was set to 1.5 N (150 kdyn), which induces moderate contusion injury. After the surgical procedure, the rats were allowed to recover on a warm blanket. From the day after the surgery, urination was assisted manually until voluntary urination was restored.

Minocycline treatment

Rats were randomly assigned into two groups ($N = 4$ per group) and were administered an intraperitoneal injection of either control saline (control group) or minocycline (15 mg ml^{-1} in saline; treated group) (Sigma, St Louis, MO, USA). The injured rats received an initial dose of 90 mg kg^{-1} body weight minocycline immediately after the injury, followed 9 h later by a second dose of 45 mg kg^{-1} body weight. From the following day, either control saline or 45 mg kg^{-1} body weight minocycline was administered twice a day up to 3 days post injury (dpi).[12,14]

To evaluate the motor recovery, animals were observed by one individual masked to the treatments on 1, 3, 7, 14, 21 and 28 dpi. Lower limb functions were assessed by the Basso, Beattie and Bresnahan scale.[15]

Measurement of blood pNF-H levels

Blood samples were taken from the tail vein of the injured rats on 1, 2, 3, and 4 dpi. The blood samples were anticoagulated using ethylenediamine tetraacetic acid and centrifuged at 3000 r.p.m. for 10 min to obtain plasma. The plasma samples were frozen and stored until the pNF-H

assay was performed. The pNF-H assay was carried out using a commercially available enzyme-linked immunosorbent assay kit (ELISA-pNF-H; EnCor Biotechnology, Gainesville, FL, USA). The frozen plasma samples were allowed to thaw, and then diluted 1/5 with a dilution buffer. The samples were then loaded onto an enzyme-linked immunosorbent assay plate. The assay was performed according to the manufacturer's protocol. To standardize the pNF-H value, we divided the absolute pNF-H concentration by the total protein concentration of each sample.

Statistical analysis

The hind limb function score was analyzed by the Mann–Whitney U-test. Quantitative plasma pNF-H values between the two groups were analyzed by repeated-measure analysis of variance. The relation between plasma pNF-H levels and hind limb function score was assessed by determining the Spearman's rank correlation coefficient. Error bars indicate the standard error, and differences with a P-value of <0.05 were considered statistically significant.

We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research.

Results

Minocycline improves hind limb motor function after incomplete spinal cord injury

To determine whether plasma pNF-H levels can be used as a marker for evaluating the efficacy of neuroprotective therapies for SCI, we analyzed the plasma pNF-H levels in experimental SCI rats treated with minocycline, a drug with proven neuroprotective effects.[3,12–14] As minocycline is known to prevent secondary damage in neural tissue by inhibiting microglial activation,[3,12] we assumed that the administration of this drug may also reduce biomarkers of tissue damage.

First, we confirmed the efficacy of minocycline treatment. Behavioral assessments at 28 dpi (Figure 1) revealed that minocycline-treated rats had better hind limb motor function than did the control rats (mean Basso, Beattie and Bresnahan score ± standard error of the mean (s.e.m.): treated group, 14.5 ± 0.6 vs control group, 12.4 ± 0.4; $P < 0.05$). The better hind limb function observed in the treated rats suggests that minocycline exerted neuroprotective effects after SCI in this rat model.

Minocycline treatment modulates plasma pNF-H levels

Because minocycline reduces axonal damage in the injured spinal cord by preventing secondary damage, we assumed that the plasma pNF-H level in the treated group would be lower than that in the control group. The plasma pNF-H level and pNF-H/total protein ratio were determined from the blood samples (Figure 2). In both the groups, pNF-H was detected from 1 dpi and its levels peaked at 3 dpi; however, the pNF-H level at 3 dpi was lower in the treated group than in the control group (treated group, $0.088 \pm 0.018\,\mu g\,g^{-1}$; control group, $0.112 \pm 0.011\,\mu g\,g^{-1}$). Although this difference
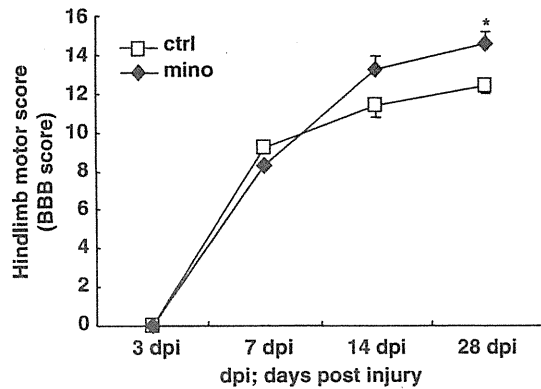
Figure 1 The functional recovery of hind limbs after spinal cord injury in both the treated and control groups. Thoracic spinal cord injured rats were treated with either minocycline (treated group: mino) or saline (control group: ctrl). The Basso, Beattie and Bresnahan (BBB) scale was used to assess the motor function of the hind limbs of the injured rats. At 28 dpi, the treated group showed better motor function, and the difference between the two groups was statistically significant (values are shown as mean ± standard error of the mean; *$P<0.05$).
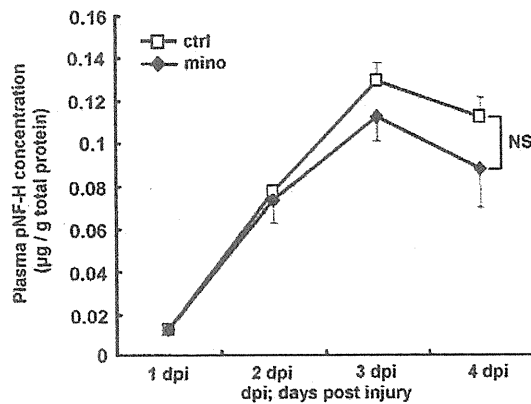

Figure 2 Changes in plasma pNF-H levels induced by minocycline treatment. The levels of plasma pNF-H in serial samples obtained from injured rats in both the treated (mino) and control (ctrl) groups were analyzed by an enzyme-linked immunosorbent assay. The absolute pNF-H values were divided by the total protein concentration of each sample (values are shown as mean ± standard error of the mean). Even though the pNF-H level in the treated group was lower than that in the control group at 3 and 4 dpi, the difference was not statistically significant.

was not statistically significant (repeated-measure analysis of variance; $P=0.27$), the same trend was observed at 4 dpi. The reduction in the plasma pNF-H levels at 3 and 4 dpi may indicate the protective effects of minocycline against axonal damage in the treated group.

Plasma pNF-H levels at 3 dpi correlate with locomotor function recovery

As the plasma pNF-H level at 3 dpi was lower in the treated group than in the control group, we next examined whether the pNF-H level at 3 dpi can serve as a predictor of the recovery of hind limb function. Figure 3 shows a plot of
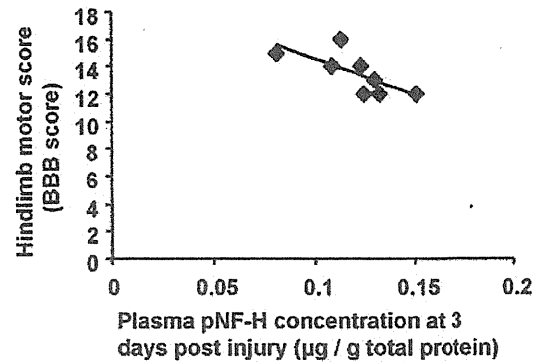

Figure 3 Correlation between plasma pNF-H level and recovery of hind limb function after spinal cord injury. The plasma pNF-H concentration at 3 dpi was plotted against the hind limb functional score at 28 dpi for each sample (both the treated and control groups). The regression line indicates a negative correlation between these two parameters.

the plasma pNF-H level (3 dpi) and hind limb motor score (28 dpi) for each rat in both groups. Statistical analysis revealed a negative correlation between these two parameters (Spearman's rank correlation coefficient: $r_s = -0.78$; $P<0.05$), indicating that a subject with a low pNF-H level is more likely to achieve a higher motor score at 28 dpi.

Discussion

This study is the first to assess whether blood pNF-H levels reflect the neuroprotective effects of minocycline against SCI. We found that minocycline treatment reduced plasma pNF-H levels at 3 dpi, and this reduction was correlated with the functional motor score; this finding is consistent with the reported neuroprotective effects of the drug. Therefore, we suggest that plasma pNF-H levels can serve as a biomarker, to some extent, for monitoring the amelioration of tissue damage by SCI treatments.

We observed a reduction in plasma pNF-H levels in the treated group at 3 dpi, but the difference between the two groups was not statistically significant. This marginal reduction in the pNF-H level could be caused by either a minimal protective effect on the axons induced by minocycline or a timing error in blood sample collection. Most of the studies on minocycline treatment for SCI describe the difference in the histological findings between the treatment and control groups at 7 or 14 dpi.[3] The plasma pNF-H level decreased after 3 dpi, and this finding was consistent with other reports.[9] Further, we speculate that the pNF-H level at later time points would be influenced by restoration of the blood–brain barrier. Therefore, the comparison of the pNF-H levels at 7 or 14 dpi would not be feasible for evaluating the neuroprotective effect of therapeutic interventions. On the basis of these findings, we consider that blood pNF-H levels would best reflect the effects of neuroprotective drugs if the drugs exert their function within 3 days after SCI.

The association between serum pNF-H levels and prognosis has already been reported in studies with subarachnoid

hemorrhage and amyotrophic lateral sclerosis patients.[10,16] To determine the utility of pNF-H as biomarker of clinical SCI, further studies with patients with varying degrees of SCI are required. Taking such biomarkers in clinical trials may reduce the number of the patients required, which accelerates the development of novel therapeutic approaches against the traumatic disorder.

Conflict of interest

The authors declare no conflict of interest.

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Robotic-assisted stepping modulates monosynaptic reflexes in forearm muscles in the human

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[1]*Motor Control Section, Department of Rehabilitation for the Movement Functions, Research Institute, National Rehabilitation Center for Persons with Disabilities, Saitama, Japan;* [2]*Rehabilitation Neuroscience Laboratory, University of Victoria, British Columbia, Canada;* [3]*Graduate School of Engineering, Shibaura Institute of Technology, Tokyo;* [4]*Graduate School of Systems and Information Engineering, University of Tsukuba, Ibaraki;* and [5]*Division of Health and Sports Sciences, Faculty of Education, Chiba University, Japan;* [6]*Motion and Mobility Laboratory, University of Victoria, British Columbia, Canada;* and [7]*Graduate School of Arts and Sciences, University of Tokyo, Japan*

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. *J Neurophysiol* 106: 1679–1687, 2011. First published July 20, 2011; doi:10.1152/jn.01049.2010.—Although the amplitude of the Hoffmann (H)-reflex in the forelimb muscles is known to be suppressed during rhythmic leg movement, it is unknown which factor plays a more important role in generating this suppression—movement-related afferent feedback or feedback related to body loading. To specifically explore the movement- and load-related afferent feedback, we investigated the modulation of the H-reflex in the *flexor carpi radialis* (FCR) muscle during robotic-assisted passive leg stepping. Passive stepping and standing were performed using a robotic gait-trainer system (Lokomat). The H-reflex in the FCR, elicited by electrical stimulation to the median nerve, was recorded at 10 different phases of the stepping cycle, as well as during quiet standing. We confirmed that the magnitude of the FCR H-reflex was suppressed significantly during passive stepping compared with during standing. The suppressive effect on the FCR H-reflex amplitude was seen at all phases of stepping, irrespective of whether the stepping was conducted with body weight loaded or unloaded. These results suggest that movement-related afferent feedback, rather than load-related afferent feedback, plays an important role in suppressing the FCR H-reflex amplitude.

flexor carpi radialis; Hoffmann reflex; *soleus* muscle; passive stepping; stepping-related feedback

DURING RHYTHMIC ARM CYCLING, Hoffmann (H)-reflex amplitude in the *soleus* muscle (Sol) is strongly suppressed in humans (Frigon et al. 2004; Hundza and Zehr 2009; Loadman and Zehr 2007). Interestingly, leg cycling also leads to suppression of the H-reflex amplitude in the forearm muscles (Zehr et al. 2007). These results suggest a reciprocally organized pattern-generating system activated by descending locomotor commands and afferent feedback that modulates reflex excitability in remote muscles (Zehr and Duysens 2004; Zehr et al. 2009). Currently, though, the neural mechanisms producing this organization remain unclear.

Loadman and Zehr (2007) suggested that central commands for rhythmic arm cycling were a major source of modulation,

because differences in the range of arm motion (i.e., range of muscle-length change) did not alter H-reflex amplitude in stationary leg muscles. A central source for the modulation is also suggested by the observation that active arm cycling induces phase-dependent modulation of the Sol H-reflex (de Ruiter et al. 2010). In addition, Hundza et al. (2008) suggested that suppression of the Sol H-reflex during passive arm cycling was small compared with during active arm cycling. These reports suggest that central drive for rhythmic arm movement is important in suppressing H-reflexes in the ankle extensor muscles. Considerably less is known about the modulation of H-reflexes in arm muscles during passive leg movement, and the underlying mechanisms require further clarification. Importantly, the extent to which modulation of reflex excitability within a limb muscle can be fully accounted for by central drive or different sources of afferent feedback is unclear. Answering this means determining the relative contributions of movement-related afferent feedback, load-related bias, and central motor commands in the amplitude modulation of H-reflexes in forearm muscles.

Phase-dependent modulation of H-reflex amplitude in arm muscles during rhythmic leg movement remains an uncertain area. Phasic modulation of the forearm flexor H-reflex was seen with isolated, rhythmic foot movement (Baldissera et al. 1998) but not with rhythmic leg cycling (Zehr et al. 2007). Currently, there are no comparable data from arm muscles during walking-based driven gait orthosis (DGO) stepping and passive movement of the leg. Since passive DGO leg movement resembles "passive" cycling as a whole-leg rhythmic locomotor movement, we hypothesized that the passive stepping would suppress H-reflex amplitudes in the forearm, irrespective of body loading and without phase-dependent modulation.

We demonstrated previously that phase-dependent, cutaneous reflex modulation was absent in leg muscles during unloaded stepping, during passive stepping, suggesting that load-related afferents were important for generating phasic modulation of the cutaneous reflex in leg muscles (Nakajima et al. 2008). However, a similar approach did not affect Sol H-reflex modulation, suggesting that the effect of loading during passive stepping strongly depends on excitability in reflex circuitry (Kamibayashi et al. 2010). This parallels the earlier suggestion of Zehr et al. (2001), showing a differential mod-

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ulation of cutaneous and H-reflexes in leg muscles depending on motor output and loading during leg cycling. However, it is not known whether lumbar load-related afferent feedback modulates the excitability of monosynaptic H-reflex circuits in the cervical spinal cord. We hypothesized that although we can expect a modulation in H-reflex amplitude in the remote limb, loading should have only a small effect on phase-modulation during DGO passive stepping. Absence of a modulatory effect of load-related afferent feedback on H-reflex excitability would implicate movement-related afferents in contributing to the pattern of H-reflex modulation observed during passive leg movement.

MATERIALS AND METHODS

Subjects

Participants were 16 healthy males, aged 22–32 yrs. All gave informed, written consent prior to participation in the experiments. The protocol was approved by the local ethics committee of the National Rehabilitation Center for Persons with Disabilities (Saitama, Japan) and is in accordance with the guidelines set out in the Declaration of Helsinki (1964).

General Procedure

The recently developed DGO system, Lokomat (Hocoma AG, Volketswil, Switzerland), was used to produce "passive stepping", defined as stepping movements driven by the DGO system while relaxing the leg muscles. In this paradigm, there may be some very low-level, incidental muscle activation, but it is involuntary (see Kamibayashi et al. 2009). A detailed description of the Lokomat can be found elsewhere (Colombo et al. 2001). Briefly, this system consists of a treadmill, a body-weight support system, and two robotic actuators that are attached to each subject's legs. The Lokomat is fully programmable, including the control of knee and hip kinematic trajectories during different types of stepping, with and without body-weight loading.

The right forearm, wrist, and hand were fixed to a rigid platform to minimize any unwanted movement of the arm. A brace was worn to restrict arm movement and was fixed on the elbow and wrist positions at 90° and 0°, respectively, in all experiments. All trials were performed when the *flexor carpi radialis* (FCR) muscle was quiescent. Unloading of body weight was accomplished by suspending the subject's body with a harness connected to an overhead crane. For all passive stepping conditions, the treadmill speed was kept constant at 2.0 km/h for all subjects. During passive stepping, the subjects were instructed to relax and allow the lower-limb movements to be imposed by the DGO. Dorsiflexion of the ankle joint during the stepping condition was achieved by passive foot lifters (spring-assisted elastic straps) to prevent foot drop at the swing phase (see Kamibayashi et al. 2010).

Experimental Tasks

To explore the effect of passive leg stepping on the upper arm H-reflex amplitude, subjects participated in three experiments using the Lokomat systems: *1*) phase-modulation of the FCR H-reflex during passive stepping (*n* = 10); *2*) determination of the H-reflex and muscle response (M-wave; H-M) recruitment curves during passive stepping (*n* = 8; all participated in *experiment 1*); and *3*) effects of loading on the FCR H-reflex during passive stepping (*n* = 10). Of these last 10, four participated in one or both of the other experiments, and six participated only in this experiment. When a given subject participated in two or three experiments, data from each experiment were collected on different days. Based on a previous study (Javan

and Zehr 2008), the intervening time interval is sufficient for residual suppression to disappear; thus our results across experiments were not affected by residual suppression. During experiments to investigate phase-modulation and the effect of loading on FCR H-reflex amplitudes, experimental conditions (phases or load conditions) were pseudo-randomly executed for each subject.

Effect of stepping-related afferent feedback on FCR H-reflex. In 10 subjects, the effect of leg stepping-related afferent feedback on the FCR H-reflex amplitude was investigated during robotic-assisted passive stepping and standing conditions (40% unloading of body weight). The subjects performed passive stepping on the treadmill with the arms at rest. Electrical stimulations to elicit the H-reflex were pseudo-randomly delivered at 10 different phases of the stepping tasks (see *FCR H-reflexes* below).

Effect of load-related afferent feedback on FCR H-reflex during DGO stepping. To investigate the effect of load-related afferent feedback on the FCR H-reflex during passive stepping, 10 subjects performed passive stepping and standing in separate trials under two conditions: *1*) unloaded condition (100% of body-weight support) above the treadmill belt and *2*) loaded condition (40% unloading of body weight) on the treadmill (Kamibayashi et al. 2009, 2010). Reflexes were evoked at the midstance phase and during standing, with and without loading of body weight for all trials.

FCR H-Reflexes

FCR H-reflexes in the right arm were evoked by stimulating (rectangular pulse, 0.5-ms duration) the median nerve with a constant current electrical stimulator (SEN-7023, Nihon Kohden, Tokyo, Japan). Bipolar stimulus electrodes were placed just proximal to the medial epicondyle of the humerus, near the *cubital fossa* (cf. Zehr et al. 2007). To examine the effect of leg position on FCR H-reflex in the passive stepping, the step-cycle duration was set at ~2.0 s by the Lokomat system and divided into 10 phases. The step duration was chosen to ensure subjects' safety and effectively avoid unintentional electromyographic (EMG) activity of the leg muscles. The electrical stimulations were pseudo-randomly delivered at various times during the stepping tasks (0, 200, 400, 600, 800, 1,000, 1,200, 1,400, 1,600, and 1,800 ms after the trigger signal), defined by predetermined hip-joint angles. The trigger signal was made by hip angles coincident with the timing of heel contact. These stimuli were delivered randomly once every two to three step cycles. The stimulus intensity was adjusted to ~10% of the maximal amplitude of the direct motor response (M_{max}) in each phase (cf. Kamibayashi et al. 2010; Simonsen and Dyhre-Poulsen 1999). These stimulation intensities were confined to H-reflex amplitudes evoked on the ascending limb of the recruitment curve of the H-reflex. The consistency of the test stimulus was confirmed by examining the shape and peak-to-peak amplitude of the M-wave. As controls, M_{max} (*n* = 5 sweeps) and H-reflex (*n* = 12 sweeps) amplitudes were measured during standing and at each of the 10 stepping phases.

In additional control experiments, the H-M recruitment curves were recorded in eight subjects during quiet standing and at the stance and swing phases of passive stepping. The stimulus intensity was increased gradually from below the threshold of the H-reflex to supramaximum stimulation of the M-wave. Five responses were recorded at each of the stimulus intensities.

To investigate the effect of load-related afferent feedback on the FCR H-reflex amplitude during passive stepping, recruitment curves were also recorded during standing and at the stance phase of passive stepping, with and without loading of body weight in 10 subjects. All trials were performed when FCR was quiescent.

EMG Recording

EMG activity was recorded from the FCR, *extensor carpi radialis* (ECR), *rectus femoris* (RF), *biceps femoris* (BF), *tibialis anterior*

(TA), and Sol muscles on the right side. EMG signals were obtained with surface electrodes (SS-2096, Nihon Kohden) over the belly of each muscle after reducing skin impedance (below 10 kΩ) by light abrasion and alcohol cleaning. All EMG signals were amplified ($\times$1,000) and band-pass filtered between 15 Hz and 3 kHz via a bioamplifier system (MEG-6108, Nihon Kohden). All EMG and angular signals were converted to digital data with an analog-to-digital converter card [Micro1401, Cambridge Electronic Design (CED), Cambridge, UK] and stored on a hard disk with a sampling rate of 5 kHz using Spike2 software (CED).

Data Analysis

Peak-to-peak amplitudes of M-waves and H-reflexes were normalized to the respective M_{max} amplitudes recorded during standing and at each phase of stepping.

Analysis of H-M recruitment curve. H-reflex amplitudes from the standing control curves were compared with those from the same values induced by electrical stimulation on the curves conditioned by stepping at midswing and midstance (Klimsta and Zehr 2008; Mezzarane et al. 2011; Zehr et al. 2007). Stimulus intensities for eliciting the maximum H-reflex (H_{max}) and ~50% H_{max} values were defined from the recruitment curves obtained during the standing control. For the H-M recruitment curve, means and SDs were calculated and plotted with respect to the intensity of electrical stimulation (recruitment curve; see Figs. 5 and 7).

Reflex amplitudes of the standing, midswing, and midstance phases of stepping, obtained by two stimulus strengths for eliciting ~100% and ~50% of the H_{max} at control, were compared using two-way repeated measures (RM) ANOVA (three conditions $\times$ two stimulus intensities). Two-way RM ANOVA was also used to examine modulation of the H-reflex amplitudes, with and without loading at the stance phase of passive stepping and during standing (two load conditions $\times$ two tasks).

Analysis of the FCR H-reflex amplitude at the 10 stepping phases and during standing. The H-reflex, M-wave in the FCR and background (BG) EMG activities in the FCR, ECR, RF, BF, Sol, and TA were compared using a one-way RM ANOVA [factors; standing control and passive stepping (10 phases of step cycle)]. The BG EMG activity was calculated as the root mean square value of the EMG signal for 50 ms before the electrical stimulation. Multiple comparisons were performed using the Bonferroni post hoc test. The data were expressed as means $\pm$ SEM. Significant differences were recognized at $P < 0.05$ in all cases. All statistical tests were performed using SPSS software version 11.0 (SPSS, Chicago, IL). The F values and degrees of freedom were obtained after Greenhouse-Geisser correction when appropriate.

RESULTS

EMG and Kinematic Patterns during Passive Stepping

Figure 1 shows typical recordings of EMG activities and joint angles during passive stepping (40% unloading of body
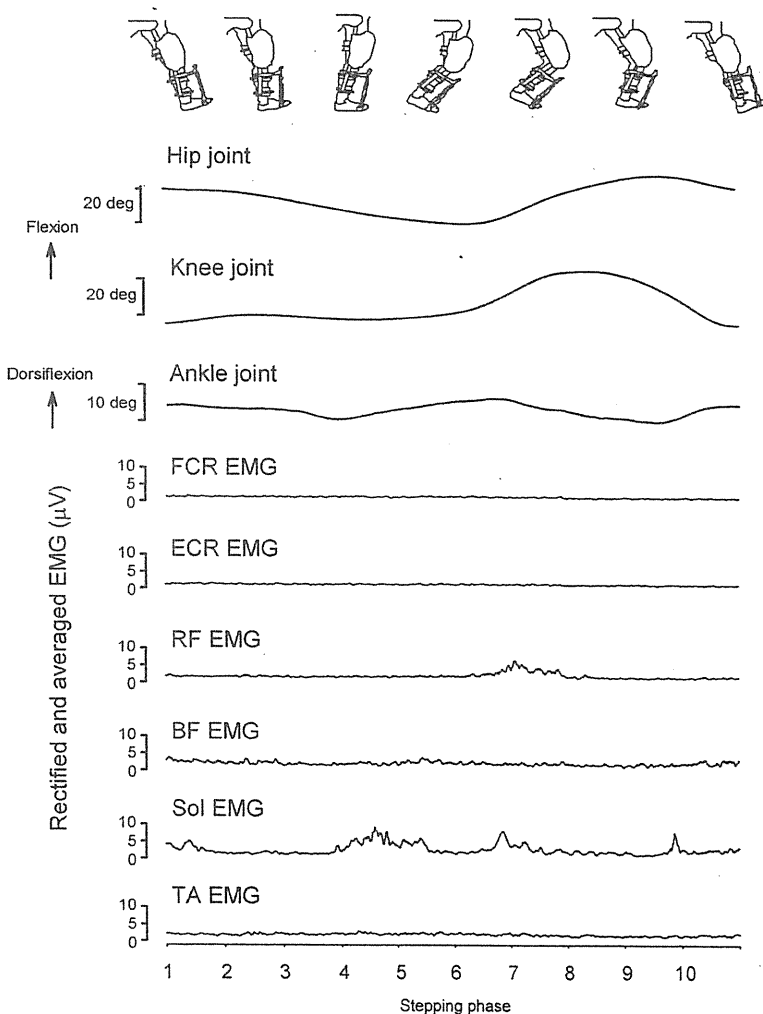

Fig. 1. Typical averaged recordings of joint angle and electromyographic (EMG) activity in the *flexor carpi radialis* (FCR), *extensor carpi radialis* (ECR), *biceps femoris* (BF), *rectus femoris* (RF), *soleus* (Sol), and *tibialis anterior* (TA) muscles obtained from a single subject during driven gait orthosis (DGO) passive stepping. EMG data were full-wave rectified and averaged (12 sweeps). Tracings of the motion of the lower limbare, which were synchronized to the stepping cycle and the joint and EMG data, are shown at the top of the figure.

73

weight) for a single subject. Because the hip- and knee-joint trajectories were controlled by the robotic-assisted DGO system, joint movements were highly reproducible. Also, the trajectory of the ankle joint was modulated during passive stepping. EMG activities of the FCR, ECR, BF, and TA were quiescent during passive stepping, whereas those of the Sol and RF EMG activities were slightly visible in several stepping phases of this subject.

Figure 2 illustrates the group means of the BG EMG activities obtained from 10 subjects during passive stepping and static conditions. Although the amplitudes of the FCR, ECR, BF, RF, and TA did not change and did not differ significantly across phases and standing conditions [one-way ANOVA: FCR, $F^{(10,90)} = 1.298$, $P > 0.05$; ECR, $F^{(10,90)} = 1.119$, $P > 0.05$; BF, $F^{(10,90)} = 1.179$, $P > 0.05$; RF, $F^{(10,90)} = 0.639$, $P > 0.05$; TA, $F^{(10,90)} = 0.708$, $P > 0.05$], the mean amplitude of the Sol EMG was significantly larger at *phase 5* than during other phases [one-way ANOVA: Sol, $F^{(10,90)} = 3.055$, $P < 0.05$; Bonfferoni post hoc: *phases 2, 7, 8, 9*, and *10*, $P < 0.05$]. However, there was no significant difference between standing and *phase 5* (Bonfferoni post hoc, $P > 0.05$).

Modulation of FCR H-Reflex Amplitude during Passive Stepping

Figure 3 shows representative recordings of the FCR H-reflex during standing and at different phases of passive stepping obtained from a single subject. The amplitude of the FCR H-reflex was suppressed strongly during stepping compared with the standing condition. The suppressive effect on the FCR H-reflex amplitude was seen at all phases of stepping, with little difference based on phase. Figure 4, *A* and *B*, illustrates pooled data for the amplitudes of the FCR H-reflex and

M-wave, respectively, obtained from 10 subjects during passive stepping and static standing. Although the amplitudes of the M-wave and BG EMG activities did not differ significantly across phases [compare Fig. 4*B* with Fig. 2*A*; one-way ANOVA: M-wave, $F^{(10,90)} = 0.621$, $P > 0.05$], the mean amplitude of the FCR H-reflex during passive stepping was significantly smaller than those during standing (Fig. 4*A*; Bonferroni test, $P < 0.001$). The one-way RM ANOVA of H-reflex amplitudes revealed a significant main effect for conditions [$F^{(10,90)} = 13.152$, $P < 0.001$]. However, there was no significant difference in H-reflex amplitudes across the stepping phases (Bonferroni test, $P > 0.05$). M_{max} amplitude did not change significantly during the static standing condition and the 10 stepping phases [one-way ANOVA: $F^{(10,90)} = 0.26$, $P > 0.05$].

Figure 5 shows the H-M recruitment curves during standing and at the stance and swing phases of passive stepping obtained from a single subject. It is notable that passive stepping reduced H-reflex amplitudes in the FCR across a wide range of stimulus strengths. Similar results were obtained from eight subjects. Interestingly, the extent of the H-reflex suppression did not depend on the size of the control H-reflex. Figure 6 illustrates the group means of the H-reflex amplitude ($n = 8$) obtained with two different stimulus strengths (i.e., for eliciting the H_{max} amplitude and ~50% of H_{max} during the standing condition). There was a significant suppression of H-reflex amplitude during both the stance and swing phases (Bonferroni test, $P < 0.05$). The two-way RM ANOVA showed a significant main effect and interaction [condition: $F^{(2,14)} = 21.008$, $P < 0.001$; stimulus intensity: $F^{(1,7)} = 31.360$, $P < 0.001$; condition × stimulus intensity: $F^{(2,14)} = 6.718$, $P < 0.01$].

Fig. 2. Grand means ($\pm$SEM) of the amplitudes of background EMG activity in the FCR, ECR, BF, RF, Sol, and TA muscles obtained from 10 subjects during standing (open circles) and DGO passive stepping (10 phases; filled circles) conditions (40% unloading of body weight). *$P < 0.05$; **$P < 0.01$.
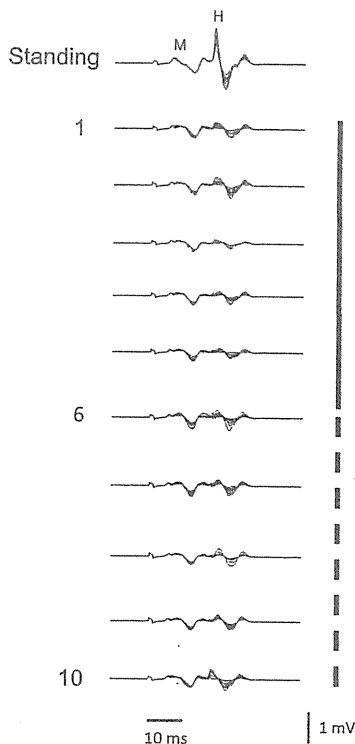
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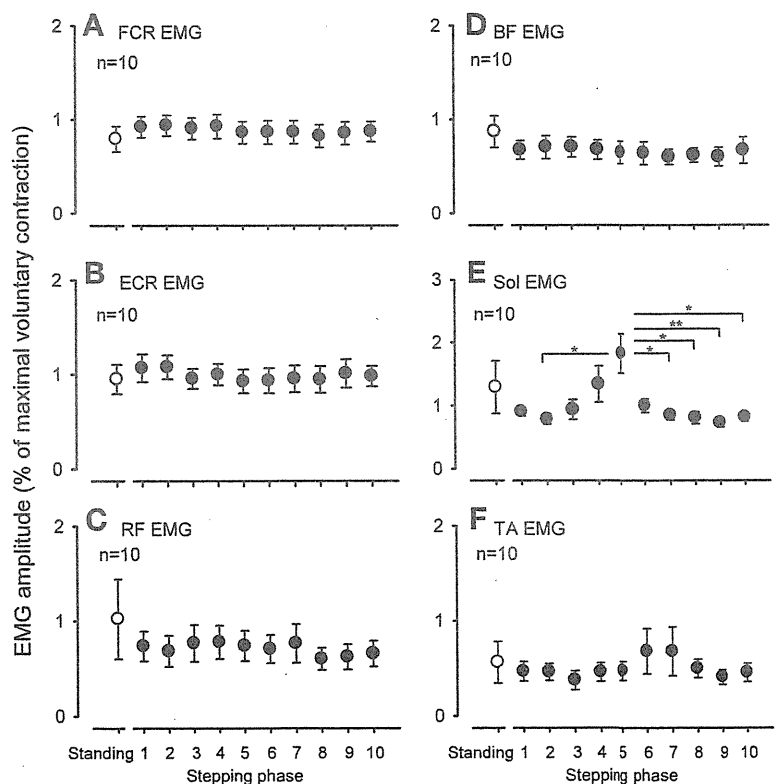
Fig. 3. Typical superimposed recordings (12 sweeps) of FCR Hoffmann (H)-reflex waveforms (H) with a muscle response (M-wave; M) size of ~10% maximal amplitude of the direct motor response (M_{max}) at standing and at 10 phases of DGO passive stepping in 1 subject. Thick, vertical solid and dashed lines indicate stance and swing phases, respectively.

Effect of Load-Related Afferent Feedback on FCR H-Reflex Amplitude during Passive Stepping

We recorded FCR H-reflexes during the stance phase of passive stepping and standing with load (40% unloading of body weight) and with full body-weight support (100% unloading) in 10 subjects. Figure 7, A–D, depicts the H-M recruitment curves obtained from a single subject. In both the loaded and unloaded conditions, passive stepping reduced H-reflex amplitudes in the FCR across a wide range of stimulus strengths. Figure 7, E and F, shows the group means of the amplitudes of H-reflexes during the loaded and unloaded conditions, elicited by two different stimulus strengths—one for eliciting the H_{max} amplitude and the other for ~50% of the H_{max} at control standing. The FCR H-reflex amplitudes at the stance phase, with and without load, were significantly suppressed compared with the respective standing controls (Bonferroni test, $P < 0.05$). The two-way RM ANOVA showed a significant main effect of condition [load: $F^{(1,9)} = 0.095$, $P > 0.05$; condition: $F^{(1,9)} = 31.386$, $P < 0.001$; load × condition: $F^{(1,9)} = 0.717$, $P > 0.05$]; however, there was no significant difference in the reflex amplitudes between the loaded and unloaded conditions.

DISCUSSION

In the present study, we demonstrated that the magnitude of the FCR H-reflex was strongly suppressed during robotic-assisted, passive stepping, compared with that elicited during standing. The suppressive effect on the FCR H-reflex ampli-

tude was seen at all phases of stepping; however, there were no significant phase-dependent differences. Furthermore, the H-reflex amplitudes were suppressed during stepping tasks, both with and without load, with no significant effect of loading itself. These findings suggest that movement-related afferent feedback, rather than load-related afferent feedback, plays a key role in modulating the FCR H-reflex amplitude.

Methodological Considerations

In the current study, the amplitude of the direct M-wave elicited in the FCR was used as an indication of the constancy of the afferent test volley. Similar M-wave amplitudes maintained for all conditions (~10% of maximal M-wave amplitude) are an indication that the activated afferent volley evoked by the various test conditions also remains constant (Fukushima et al. 1982). In fact, there were no significant differences in the M-wave amplitudes among the 10 stepping phases and the standing condition (see Fig. 4B).

Although the H-reflex and M-wave amplitudes were normalized to the M_{max} amplitude to mitigate intersubject variability, there is a possibility that the M_{max} amplitude itself differed among step phases (Simonsen and Dyhre-Poulsen 1999) and over the time course of an experiment (Crone et al. 1999). Therefore, the M_{max} in the FCR was recorded at each stepping phase, and predetermined M-wave amplitudes were checked and adjusted carefully with respect to the M_{max} amplitude in each phase. We confirmed that there were no significant differences in the normalized M-wave amplitude during tasks. Thus the suppression of the H-reflex amplitude during passive stepping was not due to changes in the efficacy of the electrical stimulation delivered to the median nerve.
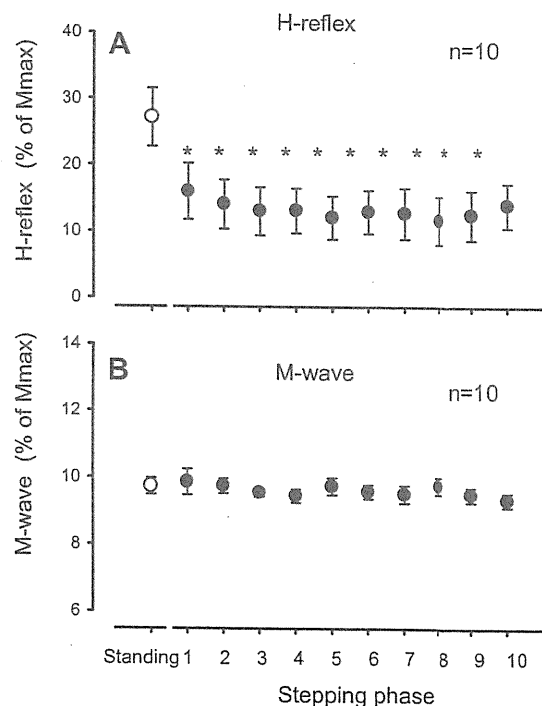

Fig. 4. Grand means (±SEM) of the magnitude of the H-reflex (A) and M-wave (B) in the FCR muscle obtained from 10 subjects during standing (open circles) and DGO passive stepping (10 phases; filled circles) conditions (40% unloading of body weight). *$P < 0.01$.
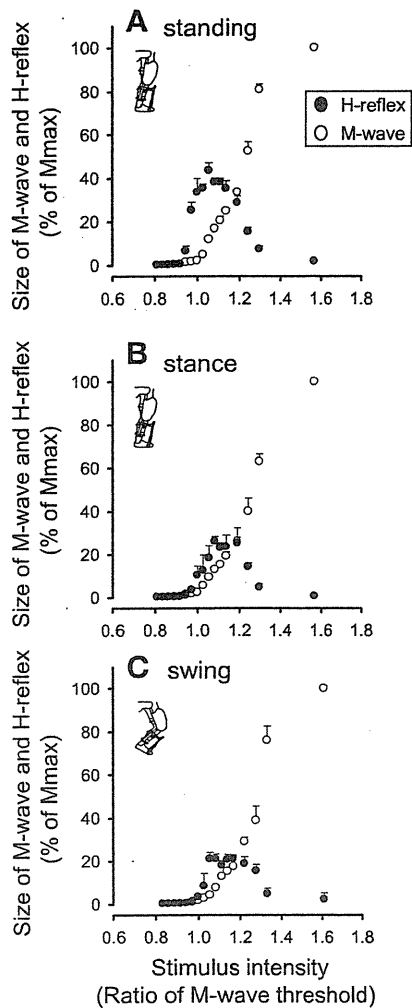
75

Fig. 5. H-reflex and M-wave (H-M) recruitment curves during standing (A) and at the stance (B) and swing (C) phases of stepping from a single subject. Each plot (H-reflexes, closed circles; M-waves, open circles) shows the mean value (+SD) of 5 responses at each stimulus intensity. Abscissa shows the intensity of the electrical stimulation with respect to the M-wave threshold.

In the present study, intensity of the test electrical stimulation to the median nerve was maintained at $\sim10\%$ M_{max} in each subject. However, this procedure inevitably required us to use different sizes of test H-reflexes depending on the subject. As it was reported that the degree of the conditioning effect on the H-reflex is proportional to the size of the test H-reflex, it is possible that the responses were affected by the size of the test H-reflex (cf. Crone et al. 1990). However, the H-reflex amplitudes evoked by a wide range of stimulus intensities were suppressed during both the stance and swing phases of passive stepping compared with those during quiet standing (see Fig. 5). Furthermore, when two different stimulus intensities ($\sim50\%$ H_{max} and H_{max} during control standing) to the median nerve were investigated, there was a significant suppression of H-reflex amplitudes at both the stance and swing phases of passive stepping for both stimulus intensities (see Fig. 6). Thus it is likely that the extent of amplitude suppression did not depend on the size of the test H-reflex (cf. Crone et al. 1990).

Reciprocal inhibitory effects arising from the forearm extensor muscles may possibly also affect the amplitude of the FCR H-reflex (Day et al. 1984). However, the amplitude of the ECR EMG activity was kept to a minimum (see Fig. 2B), and there were no significant differences across conditions during our DGO stepping and static standing. Thus suppression of the FCR H-reflex during passive stepping cannot be ascribed to a change in antagonist muscle activity.

Possible Sources of the FCR H-Reflex Suppression during Passive Stepping

Our finding that passive stepping suppressed the magnitude of the forearm H-reflex is well in line with previous reports (Frigon et al. 2004; Loadman and Zehr 2007; Zehr et al. 2007) of conditioning by remote rhythmic movement. However, one possible discrepancy between our study and previous ones is the possible substantial contribution of the voluntary drive to maintain rhythmic leg movements. Although subjects were instructed to relax and allow the DGO to drive lower-limb movements, complete passive stepping was difficult to achieve. In fact, slight Sol EMG activities [~1–2% of maximal voluntary contraction (MVC)] in the late stance phase were found and were significantly larger than those during other phases (see Figs. 1 and 2). These small Sol EMG activities during loaded stepping were also observed in our recent studies (Kamibayashi et al. 2009, 2010), even though the subjects were asked to relax. In addition, the ankle joint was held by foot lifters (spring-assisted elastic straps) to prevent foot drop and thereby restricting the trajectory of the ankle joint. Under this situation, an increase in EMG activity in the Sol at phase 5 might be a stretch-induced muscle activity, signifying that they are involuntary in nature. During normal walking, generally, it has been demonstrated that the peak EMG value of the Sol muscle was above 80% of MVC (Arsenault et al. 1986; Nishijima et al. 2010). In our situation, leg EMG activities (~0.5–2% of MVC) during DGO stepping were extremely low compared with during normal walking. Also, reciprocal EMG activity in TA was barely discernible (see Figs. 1 and 2). Therefore, it may be that the contribution of descending commands to suppression of the FCR H-reflex during DGO-driven stepping was extremely small compared with during normal
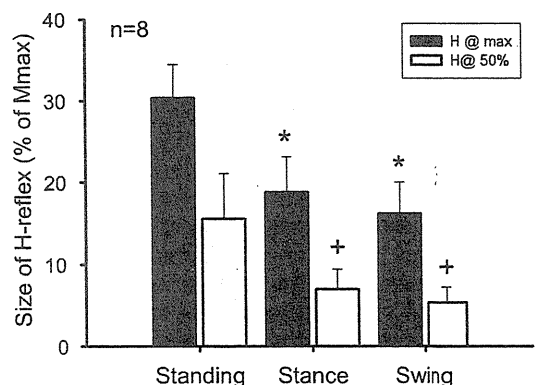

Fig. 6. Group means of the H-reflex amplitudes during standing and the stance and swing phases of passive stepping. Two different intensities of electrical stimulation were used—the intensity that elicited the maximal H-reflex (H_{max}) amplitude (black bars) and that elicited an amplitude of $\sim50\%$ of H_{max} (gray bars) during standing. *, $+P < 0.05$, significantly different from the respective control (standing) values.
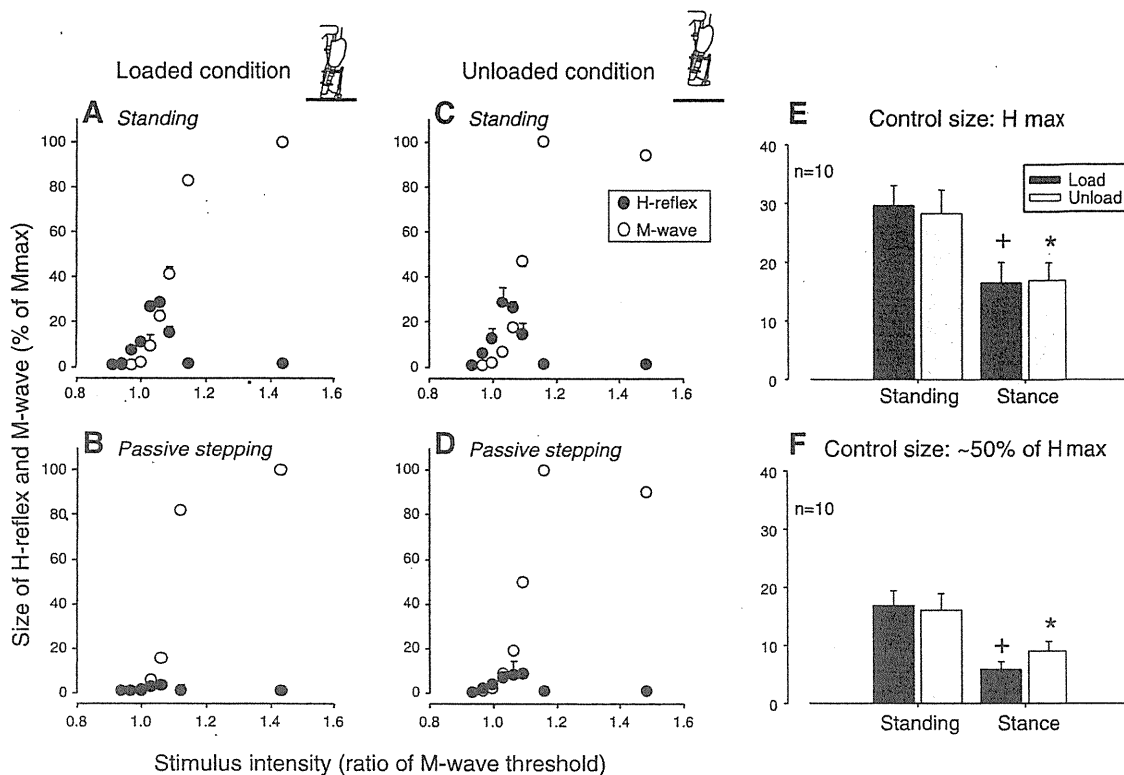
Fig. 7. Effect of loading on the FCR H-reflexes during standing and DGO passive stepping in 10 subjects. H-M recruitment curves at standing (A and C) and passive stepping (B and D), with load (40% unloading of body weight; A and B) and without load (100% unloading; C and D) obtained from a single subject. E and F: group means of H-reflex amplitudes with (black bars) and without (gray bars) load. Two different stimulus strengths were used to elicit the H_{max} amplitude (E) and ~50% of H_{max} (F) while standing. *, +P < 0.05, significantly different from values during the respective standing conditions.

walking. Furthermore, even while performing isolated knee or hip passive movements of the ipsilateral leg with the same DGO system, the magnitude of the FCR H-reflex did not show any suppression compared with during quiet standing (T. Nakajima and T. Kitamura, unpublished observations). Delwaide and Toulouse (1981) previously demonstrated that stretch reflex amplitudes in the leg muscle were not suppressed by passive, discrete wrist movement in the remote muscle. Taking all of these observations into consideration, we favor the explanation that stepping-related afferents arising from combined joint movements in both legs play a key role in generating suppression of the FCR H-reflex amplitudes during our DGO stepping.

We further investigated the effect of load-related afferent feedback on the FCR H-reflex amplitude during the stance phase of passive stepping and standing. During locomotion, inputs from load-related receptors are important for the neural control of locomotion (Dietz et al. 2002; Duysens et al. 2000; Pearson and Collins 1993; Shoji et al. 2005; Stephens and Yang 1999; Van de Crommert et al. 1998). The potential mechanoreceptors include those in muscles, skin, and joints from both legs (Duysens et al. 2000). In fact, we have observed strong facilitation of the cutaneous reflex in the TA muscle during the late-stance to early-swing phase of passive loaded stepping but not during passive unloaded stepping (Nakajima et al. 2008). More recently, we investigated the effect of body load on the amplitude of the H-reflex in the Sol muscle using the same DGO system (Kamibayashi et al. 2010) and found that the Sol H-reflex

was equally suppressed by passive stepping, both with and without body loading. This was also true in the current study for the suppression of the FCR H-reflex during passive stepping (see Fig. 6). In addition, load-related afferent feedback during rhythmic arm movement was shown to have no influence on Sol H-reflex suppression in the stationary leg (S. R. Hundza, G. C. de Ruiter, and E. P. Zehr, unpublished observations).

Based on these findings, it is unlikely that load-related afferent feedback contributed to the suppression of FCR H-reflex amplitude during passive stepping in our experimental situations [e.g., our population of subjects and number of subjects (n = 10)].

Lack of Phasic Reflex Modulation in the FCR during Passive Stepping

We found that movement-related afferent feedback from both legs does not produce a phase-dependent modulation of the FCR H-reflex (see Figs. 3 and 4). Indeed, the specific source of the modulation is not certain, although these findings may indicate that the phasic afferent inputs arising from passive leg movements are conveyed to the ascending, long propriospinal neurons responsible for the inhibition of the monosynaptic reflex arc in the other segments of the spinal cord (Alstermark et al. 1987; Cheng et al. 1998; Dietz 2002; Frigon et al. 2004; Loadman and Zehr 2007; Misiaszek et al. 1998; Zehr and Duysens 2004). More recently, de Ruiter et al. (2010) reported that suppression of the Sol H-reflex amplitude

77

was dependent on the phase of movement during active arm cycling. Probably descending commands accompanying active arm cycling generate phasic modulation of the H-reflex during remote rhythmic movement. Based on these and our findings, it is likely that afferent information for stepping plays an important role in generating tonic suppression of the H-reflex amplitude in remote muscles (Cheng et al. 1998; Misiaszek et al. 1998; Sasada et al. 2010). These features of the general suppression of forelimb reflex excitability during passive leg stepping can be explained by the afferent-induced presynaptic inhibition on the Ia terminals of the FCR H-reflex circuitry during locomotor activity (Cheng et al. 1998; Frigon et al. 2004; Misiaszek et al. 1998; Sasada et al. 2010; Zehr and Duysens 2004). This discussion is based on indirect evidence in humans (Frigon et al. 2004; Zehr et al. 2007), and further study is needed to determine the possible contribution of presynaptic inhibition on suppression of the H-reflex pathway during passive movement of the remote limb. In addition, further investigation is needed to elucidate the functional implication of walking-related afferent signals on remote H-reflex suppression during walking.

It has been suggested that it is possible to regain locomotor abilities after spinal cord injury with intense stepping training on a treadmill (Dietz et al. 2002; Van de Crommert et al. 1998). As a translational implication for rehabilitation, our findings suggest that there may be a potential therapeutic use for passive stepping in the management of spasticity in remote muscles after spinal cord injury and stroke (cf. Hundza et al. 2009; Zehr and Duysens 2004; Zehr et al. 2009). In fact, a relationship has been seen between spasticity and hyperexcitable reflexes (e.g., H-reflexes; Levin and Hui-Chan 1993); however, specific studies designed to test this hypothesis are needed.

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79

2011 年／第 48 回 日本リハビリテーション医学会 学術集会／千葉
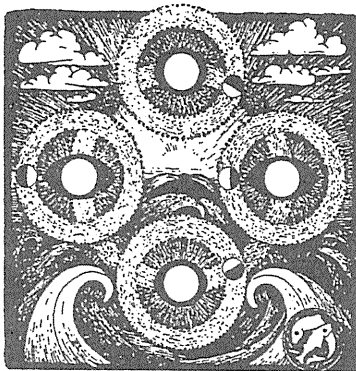
《会長講演》

物性・構造の "Manufacturing"
―人体の組織改変を人為的に起こす―*

第 48 回日本リハビリテーション医学会学術集会 会長　赤居　正美

(国立障害者リハビリテーションセンター病院・研究所)

はじめに

第 48 回日本リハビリテーション医学会学術集会開催に当たり，会長講演としてこれまでの研究内容を話す機会が与えられたので，ここに紹介させていただきたい．

私は卒業後，整形外科医として卒後研修を開始し，途中からリハビリテーション（以下，リハ）科医に移るという経歴をたどったが，基本的には運動器疾患を扱ってきた．

骨折治癒・組織修復促進の試み
―機械系としての骨・靱帯などの物性―

研究面では，整形外科ということもあり，hardware としての骨や靱帯の物性を扱うことが多かった．そこで骨折の癒合や靱帯損傷の治癒促進に関心を持ち，力学刺激や電磁場といった各種の物理刺激による組織改変をテーマに選ぶこととなった．細胞の様々な機能発現には，メカニカルストレスに代表される周囲からの物理的刺激が重要な役割を果たしているというのが前提であった．考えた作業仮説としては，①適切な刺激が加わり続けることが代謝維持に不可欠とすれば，②

外部から運動系への働きかけを用いることにより，③固定・安静による運動器官の廃用性変化の軽減・予防を目指すことができるのでは？ ④さらには機能向上につなげることも可能なのでは？ といった流れを想定した．

創外固定による脚延長や tissue expansion などのより直接的，侵襲的な手法も当時，発展しつつあったが，私の関心はもっぱら細胞組織での「刺激信号制御」を介しての間接的な方向であった．当時，骨の圧電現象や骨細管表面における流動電位などの知見もあり，動物実験を中心に電気刺激を介入手段として組織修復の促進を調べた．

外から電磁場を加えると，生体組織内では超低周波の電場が形成され，荷電粒子（イオン）の移動つまり電流を生じる．従来の報告では，周波数は概ね 300 Hz 以下，電場強度としては生体レベルで 0.1 ～ 10 V/m，細胞レベルではより微弱な 10^{-6} V/m に至る数字が挙げられる（図 1）．低周波領域での細胞膜の絶縁性は極めて高いので，外部刺激に由来する界面電位の変化はそのままでは細胞内には影響しないと考えられる．イオンチャネルやレセプターなどの膜の特定部位で何らかの透過性変化をもたらし，スイッチ機構を変えるらしい．細胞表面の局所環境の変化は，細胞膜を介して細胞内の情報伝達機構と結び付き，外因性の

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* 本稿は第 48 回日本リハビリテーション医学会学術集会会長講演をまとめたものである．（2011 年 11 月 2 日（木），幕張メッセにて）