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LETTER TO THE EDITOR

Some evidence supporting the safety of quadripulse stimulation (QPS)

To the Editor: Quadripulse transcranial magnetic stimulation (QPS) is a newly designed patterned repetitive transcranial magnetic stimulation (rTMS), which induces bidirectional, long-lasting after effects on the human motor cortex. Although QPS is a powerful tool for neurophysiologic research, there are concerns about its possible adverse effects, including induction of seizure and EEG abnormalities. In the original reports, the occurrence rates of after discharges were not significantly different between QPS and sham stimulation, and no spread of excitation was observed.[1,2] Although these studies tentatively showed the safety of QPS, further investigation is needed. In this communication, we provide further evidence of the safety of QPS comparing the vital signs, neurologic status, serum prolactin (PRL) levels, and EEGs before and after QPS.

We used eight healthy subjects aged 31 to 54 years (mean, 40.5 years) without any neurologic disorders. Before QPS, we determined the active motor threshold (AMT) of the right first dorsal interosseus (FDI) muscle by recording motor evoked potentials (MEPs; sampling rate of 10 kHz, high pass 100 Hz, low pass 3 kHz; Neuropack μ, Nihon-Kohden, Japan). TMS was given over the hot spot of FDI by a figure-of-eight coil connected to MagStim 200^2 (The MagStim Co Ltd, UK). We used QPS protocols of successive four monophasic pulses delivered with interstimulus intervals of 5 milliseconds (QPS-5) or 50 milliseconds (QPS-50). One experimental session consisted of 360 trains of four pulses (1440 pulses in total) at the intensity of 90% AMT.[1,2] Intertrain intervals were fixed at 5 seconds. QPS-5 and QPS-50 were used because previous studies reported that these protocols induced powerful potentiation and depression on the motor cortex, respectively.[1,2]

We measured vital signs in supine position for three times: before (T_{pre}), immediately after (T_0), and 180 seconds after (T_{180}) QPS. We also checked emotional state, headache, visual abnormality, dizziness, subjective hearing loss, tinnitus, aural fullness, weakness, paresthesia, and gait instability. We collected venous blood samples three times: before (T_{pre}), immediately after (T_0), and 10 minutes after (T_{10}) QPS. Because all subjects were awake from 2 hours before and throughout the experiment, the serum PRL was not affected by the sleep.[3] Serum PRL was measured with a chemiluminescence immunoassay method. Conventional EEG was recorded using a digital polygraph recording system. Ag-AgCl electrodes were attached to the scalp with conductive paste at 16 positions according to the international 10-20 system (sampling rate, 1 kHz; time constant, 0.3; low pass filter 100 Hz). EEG data were preprocessed to exclude segments with artefacts. Residual parts were divided into epochs of approximately 4 seconds (2048 points). These epochs were filtered digitally into five frequency bands from δ to γ. Approximately 100 epochs were analyzed per record.

To statistically test the effects of QPS on the blood pressure (BP), heart rate (HR), and PRL, we used two-way repeated measures analysis of variance (ANOVA) with factors of QPS-TYPE (QPS-5, QPS-50) and TIME (pre, T_0, T_{180} for BP and HR; pre, T_0, T_{10} for PRL). To examine the effects of QPS on EEG power-spectral densities, we conducted three-way ANOVA with factors of QPS-TYPE (QPS-5, QPS-50), TIMING (pre-QPS, post-QPS), and SIDE (C3, C4).

No subjects showed any emotional or neurologic symptoms during or after QPS. Hearing side effects as well as subjective symptoms were not observed. Figure 1A–C shows mean ± standard errors (SE) of systolic BP (sBP) (A), diastolic BP (dBP) (B), and HR (C) before and after QPS-5 (upper) or QPS-50 (lower). QPS-TYPE and TIME did not have significant main effects or interaction on sBP, dBP, or HR. Figure 1D shows PRL levels (mean ± SE) before and after QPS-5 (upper) or QPS-50 (lower). Neither QPS-TYPE nor TIME had significant main effect or interaction on PRL level ($P > 0.4$). Figure 1E shows percent EEG power (mean ± SE) at each frequency band recorded at C3 and C4 (filled bars, pre-QPS; bars, post-QPS). Three-ways ANOVA showed no significant main effect of QPS-TYPE, TIMING, or SIDE on any frequency band (δ, θ, α, β, γ, $P > 0.4$). None of the interactions were significant (TIMING × SIDE, TIMING × QPS-TYPE, TIMING × QPS-TYPE, SIDE × QPS-TYPE, TIMING × QPS-TYPE × SIDE, $P > 0.1$).

In summary, none of our subjects had subjective complaints as well as physical and neurologic changes.

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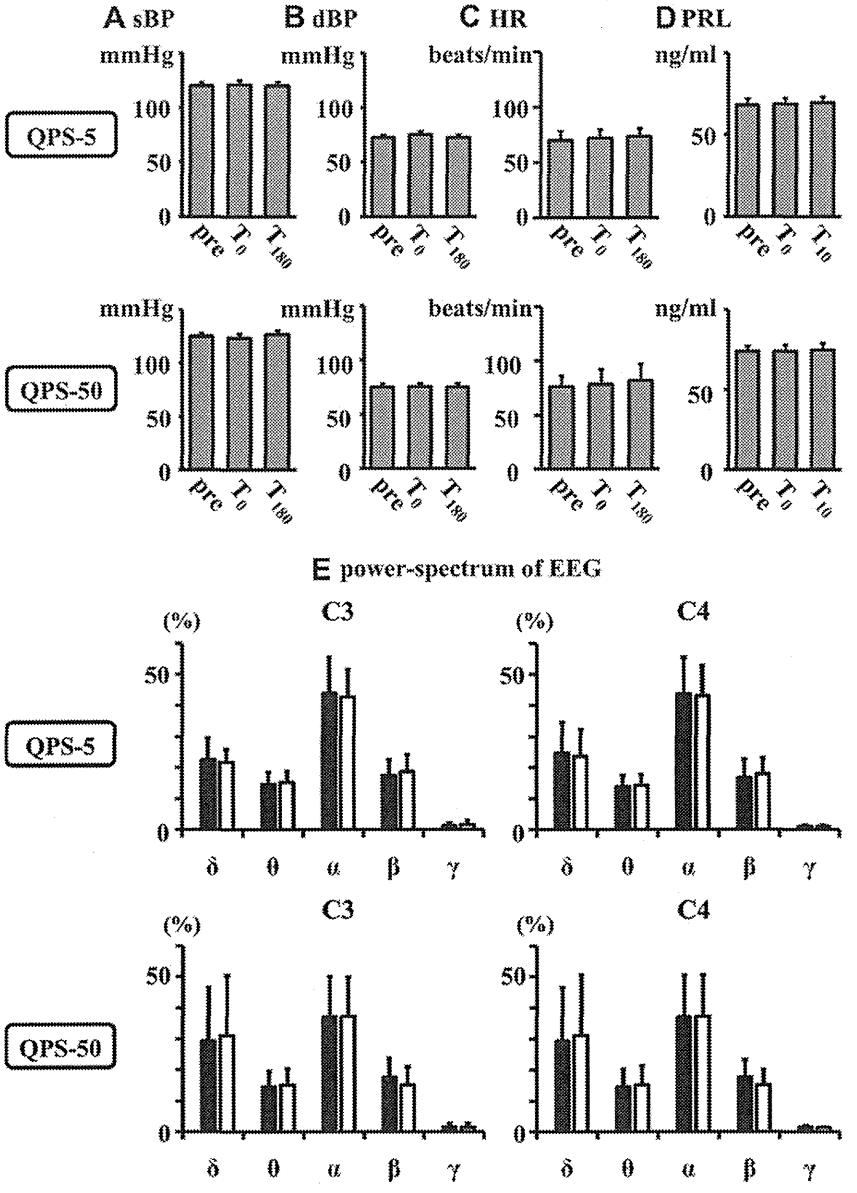

Figure 1 (**A–C**) shows mean ± SE of systolic blood pressure (**A**), diastolic blood pressure (**B**) and heart rate (**C**) before (pre), right after (T0) and 3 minutes after (T180) QPS-5 or QPS-50. There were not significant changes after QPSs in any physical parameters. Figure 1**D** shows mean ± SE of serum prolactin level before (pre), right after (T0) and 10 minutes after (T10) QPS-5 or QPS-50. They did not differ significantly. Figure 1E shows power spectrum of electroencephalogram recorded at C3 and C4 (filled bars, pre-QPS; bars, post-QPS). No any percent EEG powers were affected by QPS-5 or QPS-50.

QPS did not alter serum PRL levels or EEG frequency patterns. The results support the safety of QPS applied on the motor cortex of normal subjects with our recommended stimulation parameters.

High frequency rTMS has 1.4% crude risk estimate in epileptic patients and less than 1% in normal subjects.[4] Furthermore, in previous reports, high-frequency rTMS using suprathreshold pulses affected BP, HR,[5] or EEG.[6,7] One of 9 normal subjects experienced seizure with increases of serum PRL level after rTMS when the stimulus intensity was extremely high.[6] The lack of these changes in the present experiment may be explained by the subthreshold stimulus intensity (90% AMT) that we used.

There are a few limitations in the current study. First, we tested the null hypothesis that QPS has no effect on our measurements with a relatively small sampling population. Although the probability of incorrectly accepting the null hypothesis was kept below 5%, the power to reject the null hypothesis will increase with more subjects examined. For example, Griškova et al.[7] examined 18 subjects and found changes of EEG power in delta band with 10 Hz rTMS. Assuming the similar effect size as in the study by

Griškova et al.,[7] the power to reject the null hypothesis would increase from 63% to 97% by increasing the subject size from 8 to 18. Thus, the safety of QPS needs to be confirmed with a study of larger number of subjects. Second, the safety of QPS on pathologic brain remains uncertain. Even with these limitations, we can conclude that our current results partly support the safety of QPS. As suggested by the safety guideline, it is advisable that licensed physician supervise the experiments with careful monitoring and life-support system during QPS experiments.[4]

Setsu Nakatani-Enomoto
Department of Neurology
School of Medicine
Fukushima Medical University
Hikarigaoka 1, Fukushima, 960-1295, Japan
E-mail address: setsu-tky@umin.ac.jp

Ritsuko Hanajima

Masashi Hamada
Department of Neurology
Graduate School of Medicine
The University of Tokyo
Tokyo, Japan

Hitoshi Mochizuki

Shunsuke Kobayashi

Hiroyuki Enomoto

Yoshihiro Sugiura
Department of Neurology
School of Medicine
Fukushima Medical University
Fukushima, Japan

Hideyuki Matsumoto
Department of Neurology
Graduate School of Medicine
The University of Tokyo
Tokyo, Japan

Toshiaki Furubayashi
Department of Neurology
School of Medicine
Fukushima Medical University
Fukushima, Japan

Yasuo Terao
Department of Neurology
Graduate School of Medicine
The University of Tokyo
Tokyo, Japan

Fumio Sato
Department of Clinical Laboratory
Graduate School of Medicine
The University of Tokyo
Tokyo, Japan

Yoshikazu Ugawa
Department of Neurology
School of Medicine
Fukushima Medical University
Fukushima, Japan

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Cerebellum
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ORIGINAL PAPER

Cerebellar Stimulation in Ataxia

Stefan Jun Groiss · Yoshikazu Ugawa

Abstract The cerebellum plays an important role in movement execution and motor control by modulation of the primary motor cortex (M1) through cerebello-thalamo-cortical connections. Transcranial magnetic stimulation (TMS) allows direct investigations of neural networks by stimulating neural structures in humans noninvasively. The motor evoked potential to single-pulse TMS of M1 is used to measure the motor cortical excitability. A conditioning stimulus over the cerebellum preceding a test stimulus of the contralateral M1 enables us to study the cerebellar regulatory functions on M1. In this brief review, we describe this cerebellar stimulation method and its usefulness as a diagnostic tool in clinical neurophysiology.

Keywords Cerebellum · TMS · Cerebellar inhibition · Ataxia

Cerebellar Inhibition

The cerebellum plays an important role in movement execution and motor control by modulation of the primary motor cortex (M1) through cerebello-thalamo-cortical connections [1]. However, physiological studies of cerebellar functions were methodologically limited in humans until the introduction of transcranial stimulation techniques. Transcranial magnetic stimulation (TMS) allows us to investigate neural networks by stimulating neural structures in humans noninvasively. The motor evoked potential (MEP) to single-pulse TMS of M1 is used to measure the motor cortical excitability. A conditioning stimulus over the cerebellum preceding a test stimulus over the contralateral M1 enables us to study the cerebellar regulatory effects on M1. In healthy subjects, cerebellar conditioning TMS reduces the amplitude of MEPs to TMS of the contralateral M1, when it precedes the test stimulus by 5 to 7 ms [2, 3]. This reduction has been termed cerebellar inhibition and is mediated through a pathway between cerebellum and M1. Purkinje cells inhibit the disynaptic dentato-thalamo-cortical facilitatory connection. Cerebellar TMS must activate Purkinje cells of the cerebellar cortex, which leads to the inhibition of M1 via dentato-thalamo-cortical connections [4, 5].

Cerebellar TMS as a Diagnostic Tool

How can this technique be used in clinical neurology? It is impossible to determine the exact location of a lesion in ataxic patients based only on neurological examination, since cerebellar ataxia is caused by a lesion anywhere within the fronto-pontine-cerebello-thalamo-cortical loop. This loop consists of the cerebellar afferent pathways and the cerebellar efferent pathways including cerebellar output fibers (Fig. 1). As described above, cerebellar TMS is supposed to reflect functions of the cerebellar efferent pathways and may therefore be useful to clinically differentiate cerebellar efferent ataxia from cerebellar afferent ataxia [5].

Patients with diseases affecting the cerebellar cortex, e. g., cerebellar cortical atrophy, spinocerebellar ataxia, multiple system atrophy—cerebellar type, or cerebellar stroke, showed impaired cerebellar inhibition [6, 7]. The dentate nucleus involvement, such as dentatorubral-pallidoluysian atrophy or Wilson's disease, also reduced

S. J. Groiss (✉) · Y. Ugawa (✉)
Department of Neurology, Fukushima Medical University,
School of Medicine,
1 Hikarigaoka,
960-1295, Fukushima, Japan
e-mail: groiss-js@umin.net
e-mail: ugawa-tky@umin.net

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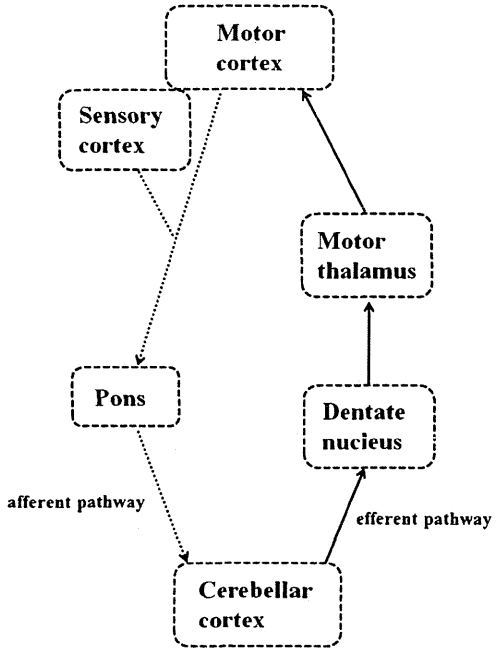

Fig. 1 Simplified scheme of the fronto-pontine-cerebello-thalamo-cortical loop. *Solid lines* indicate the cerebellar efferent pathways and *dotted lines* the cerebellar afferent pathways (modified from Kikuchi et al. [9])

cerebellar inhibition [6, 7]. In contrast, ataxic patients with the cerebellar afferent pathways involvement, such as pontine infarction or middle cerebellar peduncular involvement, had normal cerebellar inhibition. Moreover, patients without cerebellar involvement, e. g., Parkinson's disease, motor neuron disease, or peripheral neuropathy, show normal cerebellar inhibition [6, 7].

Patients with progressive supranuclear palsy (PSP) had significantly reduced cerebellar inhibition without clinically detectable cerebellar signs [8]. This is consistent with pathological and radiological findings of PSP: involvement of the cerebellar dentate nucleus and superior cerebellar peduncle. It indicates that cerebellar TMS revealed masked cerebellar dysfunction in PSP.

Ataxic hemiparesis is a lacunar syndrome with ataxia accompanying ipsilateral corticospinal tract impairment. In such patients, ataxia may result from a small lesion anywhere within the fronto-pontine-cerebello-thalamo-cortical loop. In those patients, cerebellar TMS differentiated cerebellar efferent ataxia from cerebellar afferent ataxia. Their results are consistent with the well-known anatomical knowledge of the cerebellar circuits [9].

Limitation

Cerebellar stimulation sometimes induces antidromic pyramidal tract coactivation when a suprathreshold stimulus is used. This coactivation may affect the cerebellar stimulation experiments [2, 10]. However, when the stimulation threshold is carefully defined using rectified electromyography and current direction and stimulation site are accurately and appropriately chosen, cerebellar TMS has been proven to be a powerful and reliable method to investigate cerebellar function in humans noninvasively [10].

Conclusion

Taken all these results together, cerebellar TMS is an effective and valuable method to evaluate the cerebello-thalamo-cortical loop function in humans and may be useful for pathophysiological analysis of ataxia.

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Conflict of Interest The authors report no conflict of interest related to the article.

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Cerebellum
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Ataxic Hemiparesis: Neurophysiological Analysis by Cerebellar Transcranial Magnetic Stimulation

Saeko Kikuchi · Hitoshi Mochizuki · Arata Moriya · Setsu Nakatani-Enomoto · Koichiro Nakamura · Ritsuko Hanajima · Yoshikazu Ugawa

Abstract The aim of this study was to investigate physiological mechanisms underlying ataxia in patients with ataxic hemiparesis. Subjects were three patients with ataxic hemiparesis, whose responsible lesion was located at the posterior limb of internal capsule (case 1), thalamus (case 2), or pre- and post-central gyri (case 3). Paired-pulse transcranial magnetic stimulation (TMS) technique was used to evaluate connectivity between the cerebellum and contralateral motor cortex. The conditioning cerebellar stimulus was given over the cerebellum and the test stimulus over the primary motor cortex. We studied how the conditioning stimulus modulated motor evoked potentials (MEPs) to the cortical test stimulus. In non-ataxic limbs, the cerebellar stimulus normally suppressed cortical MEPs. In ataxic limbs, the cerebellar inhibition was not elicited in patients with a lesion at the posterior limb of internal capsule (case 1) or thalamus (case 2). In contrast, normal cerebellar inhibition was elicited in the ataxic limb in a patient with a lesion at sensori-motor cortex (case 3). Lesions at the internal capsule and thalamus involved the cerebello-thalamo-cortical pathways and reduced the cerebellar suppression effect. On the other hand, a lesion at the pre- and post-central gyri should affect cortico-pontine pathway but not involve the cerebello-thalamo-cortical pathways. This lack of cerebello-talamo-cortical pathway involvement may explain normal suppression in this patient. The cerebellar TMS method can differentiate cerebellar efferent ataxic hemiparesis from cerebellar afferent ataxic hemiparesis.

Keywords Cerebellar efferent ataxic hemiparesis · Cerebellar afferent ataxic hemiparesis · Transcranial magnetic stimulation · Cerebellum

S. Kikuchi · H. Mochizuki (✉) · A. Moriya · S. Nakatani-Enomoto · K. Nakamura · Y. Ugawa
Department of Neurology, School of Medicine, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan
e-mail: mochizuki-h@umin.ac.jp

R. Hanajima
Department of Neurology, Division of Neuroscience, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Introduction

Ataxic hemiparesis (AH) is well-recognized lacunar syndrome showing homolateral ataxia with accompanying corticospinal tract impairment [1–3]. It can result from a small brain lesion positioned at various sites, such as at the pons, midbrain, internal capsule, corona radiata, and cerebral cortex [1–6].

Ataxia could be produced by lesions positioned anywhere within the cerebellar loops; cortico-ponto-cerebellar (cerebellar afferent pathways) or cerebello-thalamo-cortical projections (cerebellar efferent pathways). Several previous papers concluded that pontine or internal capsule lesions should affect the cortico-ponto-cerebellar pathways (cerebellar afferent ataxic hemiparesis) [1, 2]. Some recent studies, however, speculated that a lesion at the posterior limb of internal capsule would affect the cerebello-thalamo-cortical pathway (cerebellar efferent ataxic hemiparesis) [3–6]. These

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speculations are based on anatomical knowledge about the pathways within the cerebellar loops. The location of cerebello-thalamo-cortical pathways, however, remains to be determined at the internal capsule level [7, 8].

The interaction between the primary motor cortex (M1) and cerebellum could be evaluated by cerebellar TMS in humans [9]. In normal subjects, TMS over the cerebellum reduced motor evoked potentials (MEPs) to TMS over the contralateral M1 when it preceded the motor cortical stimulation by 5, 6, and 7 ms. This cerebellar inhibitory effect was absent or reduced in patients with a lesion involving the cerebello-thalamo-cortical pathways (cerebellar efferent ataxia), and it was normally elicited in ataxic patients with a lesion affecting cortico-ponto-cerebellar pathways (cerebellar afferent ataxia) [9]. In this communication, we applied the cerebellar stimulation to patient with AH to elucidate mechanisms for ataxia in AH.

Patients and Method

Subjects were three patients with AH who were admitted to our hospital from 2008 to 2009. All met the criteria of AH [3]: (1) new onset ipsilateral ataxia and pyramidal signs, (2) dysmetria out of proportion to the weakness, (3) absent or minimal cortical signs, and (4) all signs documented by a neurologist.

Case Presentation

Case 1

A 63-year-old woman visited our hospital because of the right side limb weakness. She showed mild hemiparesis and moderate ataxia on the right side without any sensory abnormalities. Her manual muscle test scales (MMT; Medical Research Council scale) of right side limbs were 5- to 5. Her dysmetria, dysdiadochokinesis, and terminal oscillation were out of proportion to her weakness, and Holmes-Stewart rebound phenomenon was positive.

Diffusion-weighted magnetic resonance images (DW-MRI) of the brain revealed a small infarction in the posterior limb of left internal capsule (Fig. 1a). The median nerve somatosensory evoked potential (SEPs) and central motor conduction times (CMCTs) were all within the normal range.

Case 2

A 75-year-old man visited our hospital with chief complaint of left limb sensory disturbances. He had no weakness (MMT=5) with extensor plantar response on the left side and moderate left side hemi-ataxia. Temperature and pain sensations were mildly impaired, but position and vibration sensations were normal. On the left side, his dysmetria, dysdiadochokinesis, and terminal oscillation were out of proportion to the sensory disturbances, and Holmes-Stewart rebound phenomenon was positive.

DW-MRI showed a small infarction in the right lateral thalamus (Fig. 1b). The CMCTs were all within the normal ranges, but the N20 onset-peak SEP amplitude was abnormally small in the left median nerve SEP (amplitude of N20; right median nerve 3.4 μV, left 0.2 μV).

Case 3

An 81-year-old woman was admitted to our hospital because of the left upper limb weakness (MMT=5-). She had mild monoparesis and moderate ataxia in the left upper limb. The dysmetria, dysdiadochokinesis, and terminal oscillation were out of proportion to the weakness, and Holmes-Stewart rebound phenomenon was positive.

DW-MRI showed small infarctions in the right pre- and post-central gyri (Fig. 1c). Routine examinations, bilateral CMCTs, and SEPs were all normal.

Method

Cerebellar Inhibition Induced by Cerebellar TMS

Paired TMS pulses were given over the cerebellum and the contralateral M1. The details of this method were described in our previous paper [9]. The procedures were approved by the Ethics Committee of Fukushima Medical University and performed in accordance with the 1975 Declaration of Helsinki. Written informed consent was obtained from all the subjects.

In brief, surface electromyographic activity was recorded from the target first dorsal interosseous muscle (FDI). TMS was performed with two Magstim 200 stimulators (The Magstim Co., Ltd, Whitland, UK). The conditioning magnetic stimulus was given over the cerebellum using a double-cone coil (The Magstim Co., Ltd, Whitland, UK). The center of the junction region of the coil was placed 3 cm lateral from the inion. The coil was held so that currents in the brain flowed upward. The threshold for activation of the descending motor tracts was determined as the lowest intensity eliciting five small responses (MEPs) (about 200 μV) in a series of ten stimuli when the subject made a 5% maximal voluntary contraction (about 50 μV) (threshold of case 1, 58% of maximal stimulator output; case 2, 51%; case 3, 95%) . The intensity of conditioning cerebellar stimulus was fixed at 90% of the threshold. At various times after the conditioning stimulus (ISI=5, 6, or 7 ms), the motor cortex was stimulated by a round coil placed over the vertex. The motor cortical TMS (test

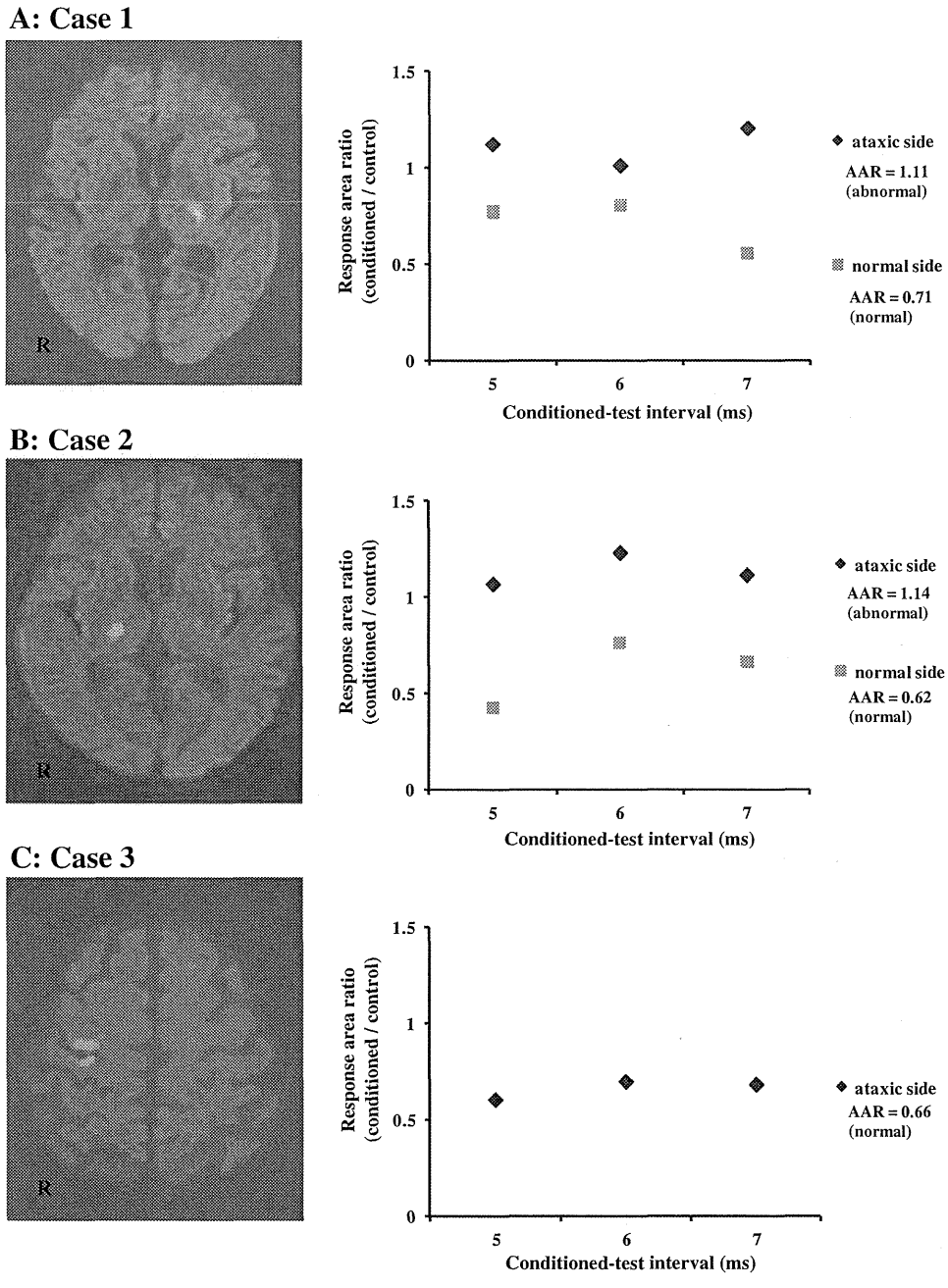

Fig. 1 *Left column* shows diffusion-weighted MRIs of the brain. *Right column* shows time courses of cerebellar suppression. Average area ratio (AAR) was indicated by each limb. *Top row* is for case 1, the *middle* for case 2, and the *bottom* for case 3. The suppression was absent in the ataxic hand in cases 1 and 2, but normal suppression was elicited in case 3. The upper limit of AAR (mean+2 SD) was 0.78 [9]

stimulus) was adjusted to produce an MEP of 0.3–0.7 mV peak to peak in the relaxed FDI when given alone.

We used a randomized conditioning-test design as reported previously [9, 10]. Various conditions (the test or conditioning stimulus given alone, or the test stimulus preceded by the conditioning stimulus by various ISIs) were intermixed randomly in one session. ISIs between the conditioning and test stimulus were 5, 6, and 7 ms. Data were analyzed off-line after the experiments. In each session, eight MEPs were collected for each condition in which both stimuli were given, and ten MEPs for the control condition in which the test stimulus was given alone. Since MEPs are often polyphasic, we routinely measured response size in terms of area of MEPs rather than peak–peak amplitude. The area of each single MEP in each condition was measured in order to compare the control and conditioned MEPs in the same session. We calculated the ratio of the mean area of the conditioned MEP to that of the control MEP for every ISI. In normal subjects, conditioned MEPs at ISIs of 5, 6, and 7 ms were suppressed. This suppression effect was considered to be absent or abnormally reduced when the average area ratio

(AAR) (ISIs=5, 6, and 7 ms) exceeded the upper limit of our normal range (mean±2SD, 0.78 [9]).

Results

Figure 1 shows brain DW-MRIs and the time courses of cerebellar suppression for three patients.

In a patient with a lesion at the posterior limb of left internal capsule (case 1), the cerebellar suppression was absent (AAR=1.11) on the ataxic side (right hand), whereas normal suppression was evoked on the normal side (non-ataxic, left hand, AAR=0.71) (Fig. 1a).

In a patient with a lesion at the right lateral thalamus (case 2), the cerebellar suppression was absent on the ataxic side (left hand) (AAR=1.14), whereas normal suppression was evoked on the normal side (non-ataxic, right hand, AAR=0.62) (Fig. 1b).

In contrast, in a patient with a lesion at the right pre- and post-central gyri (case 3), the cerebellar inhibition was normally evoked in an ataxic limb (left hand, AAR=0.66) (Fig. 1c).

Discussion

The present electrophysiological study confirmed patho-physiological mechanisms for ataxia speculated from anatomical knowledge in AH, namely cerebellar efferent AH and cerebellar afferent AH.

Previous cerebellar stimulation experiments showed that it can differentiate cerebellar efferent ataxia from cerebellar afferent ataxia physiologically [9, 11]. Figure 2 shows the supposed lesion sites for the present patients. As discussed below, anatomical knowledge suggests that the cerebellar efferent pathways (cerebello-thalamo-cortical pathways) were involved in cases 1 and 2, whereas the cerebellar afferent pathway (cortico-pont-cerebellar pathways) in case 3. The results of cerebellar stimulation (absent cerebellar suppression in cases 1 and 2, and normal suppression in case 3) support the above anatomical speculation.

Lesion Site

In 100 patients with AH, CT, or MRI analyses demonstrated that responsible lesions for ataxia were positioned at the internal capsule (39%), pons (19%), thalamus (13%), corona radiate (13%), lenticular nucleus (8%), cerebellum (4%), and frontal lobe (4%) [4]. Another study showed lesions at pons (8 cases), internal capsule (6 cases), corona radiate (2 cases), internal capsule to corona radiate (7 cases), subcortical frontal lobe (one case) or anterior central convolution (2 cases) in 25 patients with AH [5]. In our three cases, lesions were located at the posterior limb of internal capsule, lateral thalamus and pre- and post-central gyri, all of which were well-known lesion sites for AH [4, 5].

Which Part of Cerebellar Loops Was Disrupted?

Anatomical knowledge revealed that the fronto-ponto-cerebellar fibers descend through the anterior limb of internal capsule, genu, and the anterior one-third portion of the posterior limb of internal capsule [12–14]. Especially, the fibers from primary motor cortex via pontine nucleus to the cerebellum are shown to pass through the anterior one-third portion of the posterior limb of the internal capsule [14]. On the other hand, the tracts from ventro-lateral nucleus of thalamus to pericentral cortices are located at the posterior portion of the posterior limb of the internal capsule [12, 15]. The fronto-pontine pathway is positioned just anterior to the corticospinal tract which is positioned right anterior to the thalamo-motor cortical pathway. The lesion of case 1 was positioned at a posterior portion of the posterior limb of the internal capsule. Taken these all anatomical knowledge together, we speculated that the lesion would involve the thalamo-cortical fibers (cerebellar efferent AH). The lack of cerebellar suppression shown here physiologically supports this anatomical speculation. In case 2, the responsible lesion was positioned at the lateral thalamus (Fig. 2), which should affect the thalamo-cortical fibers (cerebellar efferent AH). The absent cerebellar suppression in this patient also physiologically supports this idea. In case 3, one conspicu-

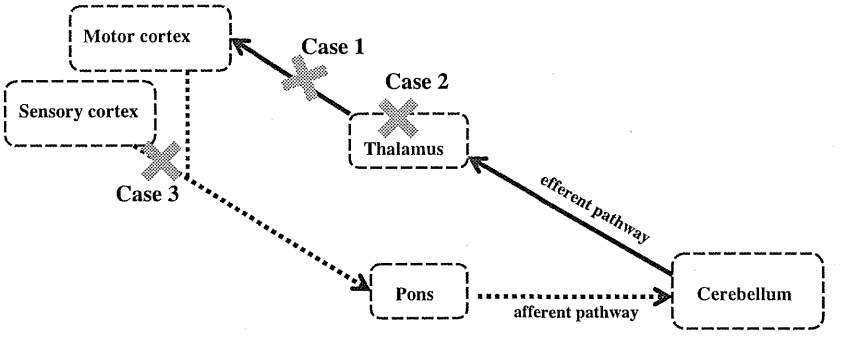

Fig. 2 The cerebellar loops. Cerebellar efferent pathways were shown by *lines* and afferent pathways by *dotted lines*. Lesion site was indicated by *crosses* for each case (×)

ous finding is that this small lesion produced apparent cerebellar ataxia at the acute phase and it faded out in a week probably because of its small size. We studied this patient when she had apparent ataxia. Based on these, we considered that cerebellar ataxia was caused by a lesion within cerebellar afferent pathways (Fig. 2). However, we could not exclude the following possibility. The small lesion must have involved a part of some cerebellar related pathway without gross impairment of cortico-pontine pathway and it caused clinical ataxia. This lesion, however, was too small to be detected by cerebellar TMS experiment. If so, our experimental results may be of no use for speculating mechanisms of the ataxia in this patient.

Conclusion

Both cerebellar efferent and afferent pathways involvements can produce ataxia in AH. Cerebellar efferent AH must be physiologically differentiated from cerebellar afferent AH by the cerebellar stimulation technique.

Conflicts of interest The authors declare that there are no potential conflicts.

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Inter-individual variation in the efficient stimulation site for magnetic brainstem stimulation

Yuichiro Shirota [a,*], Ritsuko Hanajima [a], Masashi Hamada [a], Yasuo Terao [a], Hideyuki Matsumoto [a], Ryosuke Tsutsumi [a], Shinya Ohminami [a], Toshiaki Furubayashi [b,c], Yoshikazu Ugawa [b]

[a] Department of Neurology, Division of Neuroscience, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[b] Department of Neurology, School of Medicine, Fukushima Medical University, Fukushima, Japan
[c] Department of Rehabilitation, Faculty of Medical Science and Welfare, Tohoku Bunka Gakuen University, Sendai, Japan

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HIGHLIGHTS

- We have refined the examination protocol of magnetic brainstem stimulation (BST).
- We revealed that ipsilateral stimulation elicits larger motor evoked potentials (MEPs) than the conventional inion stimulation in nearly two-thirds of healthy subjects.
- Our method will be also beneficial to the procedure of cerebellar inhibition (CBI).

ABSTRACT

Objective: To investigate inter-individual variation in the efficiency of magnetic brainstem stimulation (BST) with regard to the stimulation site.
Methods: We studied 31 healthy subjects, using a right hand muscle as a recording site. Three stimulation sites were compared: BST over the inion (inion BST), and BST over the midpoint between the inion and the right (ipsilateral BST) or left (contralateral BST) mastoid process. Five suprathreshold BSTs were performed for each stimulation site using the same stimulation intensity. The mean peak-to-peak amplitudes of motor evoked potential (MEP) were compared. The active motor threshold (AMT) and onset latency for inion BST and ipsilateral BST were also measured and compared.
Results: Contralateral BST did not evoke discernible MEPs in most subjects. In 21 subjects (67.7%), ipsilateral BST elicited larger MEPs than inion BST did, and AMT for ipsilateral BST was lower than or equal to the AMT for inion BST in all subjects. Ipsilateral BST elicited shorter latency in such subjects.
Conclusions: The suitable stimulation site for BST differed among subjects. About two-thirds showed larger MEP to ipsilateral BST.
Significance: These findings might help us to find an efficient stimulation site for BST in each subject.

1. Introduction

Transcranial magnetic stimulation (TMS) with a double-cone coil at the level of the pyramidal decussation (brainstem stimulation; BST) is clinically useful to localize corticospinal tract lesions in patients with neurological disorders (Ugawa et al., 1992, 1994, 1996). In addition, the active motor threshold (AMT) for BST, usually determined with the double-cone coil over the inion, is used as a reference when the stimulus intensity is determined for cerebellar inhibition (CBI) (Daskalakis et al., 2004; Ugawa et al., 1995).

Regarding the optimal stimulation site of BST, the inion or a point slightly below it on the midline reportedly evokes the strongest motor evoked potential (MEP) on average, but stimulation midway between the inion and the ipsilateral mastoid line also evokes strong responses (Ugawa et al., 1994). In fact, several subsequent studies showed that the optimal stimulation site for evoking responses in the first dorsal interosseous (FDI) muscle (Martin et al., 2009) or a proximal arm muscle (Butler et al., 2003; Lévénez et al., 2008) was ipsilateral to the recorded site. Nevertheless, little is known about the inter-individual difference in the suitable coil position or possible differences in AMT among stimulation sites for BST.This study investigated the variation in pyramidal tract excitability at the decussation among subjects with regard to the coil position.

* Corresponding author. Address: Department of Neurology, Division of Neuroscience, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113-8655, Japan. Tel.: +81 3 5800 8672; fax: +81 3 5800 6548.
E-mail address: yshirota-tky@umin.ac.jp (Y. Shirota).

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2. Methods

2.1. Subjects

Subjects were 31 healthy volunteers (12 women, 19 men; 26–73 years old, mean ± standard deviation (SD), 45.9 ± 15.8 years old). No subject had neurological, psychiatric, or other medical problems, or had any contra-indication to TMS (Rossi et al., 2009; Wassermann, 1998). Informed consent was obtained from all subjects. The protocol was approved by the Ethics Committee of The University of Tokyo Hospital and was conducted in accordance with the ethical standards of the Declaration of Helsinki.

2.2. Recordings

Subjects were seated on a comfortable chair. MEPs were recorded from the right FDI. Pairs of Ag/AgCl surface cup electrodes (9 mm diameter) were placed over the muscle belly (active) and the metacarpophalangeal joint of the index finger (reference). Responses were input to an amplifier (Biotop; GE Marquette Medical Systems, Japan) through filters set at 100 Hz and 3 kHz. We have used these recording conditions to reduce stimulation artifact for more than 20 years, and it is reported that MEP latency was unchanged relative to the results using a lower high-pass filter (Inomata-Terada et al., 2007); the responses were then digitized at a sampling frequency of 10 kHz, and stored in a computer for later offline analyses (TMS bistim tester; Medical Try System, Japan).

2.3. Magnetic stimulation

For BST, a double-cone coil (110 mm mean diameter, type 9902, The Magstim Co., Ltd.) was centered over the inion on the midsagittal line (inion BST), or the midpoint between the inion and the right (i.e. ipsilateral to the recording site) or left (i.e. contralateral) mastoid process, which we respectively designated as the ipsilateral and contralateral BST. Current in the coil was directed downward; that is, upward current was induced in the brain (Butler et al., 2003; Taylor and Gandevia, 2004; Ugawa et al., 1994). Although we tried inverted coil orientation in preliminary experiments (i.e. current in the coil was directed upward, so that downward current was induced in the brain), we did not obtain any responses in any of the stimulation sites described above. Monophasic TMS pulses were delivered (Magstim 200, The Magstim Co., Ltd.). All experiments were conducted with the target muscle active.

2.4. Experimental protocol

First, five suprathreshold inion BSTs were performed. We set the stimulation intensity to evoke MEPs around 1 mV. Then, five ipsilateral and five contralateral BST were applied using the same stimulus intensity. The peak-to-peak MEP amplitude and onset latency of the averaged response for each coil position was compared. The AMT for inion BST and for ipsilateral BST was determined as the lowest intensity that evoked a clear response above background electromyographic activity, which was approximately equivalent to 200 μV MEP, in more than half of consecutive trials when the subjects maintained a slight contraction of the right FDI (ca. 10% of the maximum voluntary contraction), as observed on an oscilloscope monitor. When the subject felt fatigue and considerable fluctuation of the voluntary contraction was noted, subjects were allowed to take a short break in order to restore the stability of voluntary contraction. The value of AMT was expressed as the percentage of maximum stimulator output (%MSO) in 1% steps. For contralateral BST, AMT was not measured because it did not evoke the largest MEP in any subjects.

In 20 subjects, we further compared the latency of the MEP elicited by the inion BST with that by ipsilateral BST. In this experiment, stimulus intensities were adjusted to elicit 1 mV MEP for both inion and ipsilateral BST because the amplitude of MEP reportedly influences the latency (Taylor and Gandevia, 2004). Spinal motor root stimulation was performed in the conventional manner (Chen et al., 2008; Matsumoto et al., 2010). Spinal conduction time was calculated by subtracting the root stimulation latency from the BST latency for each BST site.

2.5. Data analyses

The MEP amplitudes elicited by inion BST and those by ipsilateral BST were compared using paired *t* tests. The MEPs to contralateral BST were not analyzed because contralateral BST did not evoke clear MEPs in most subjects. First, data from all subjects were analyzed together. For analyses of AMT, the subjects were divided into two sub-groups with regard to MEP amplitude: those who had larger MEP with inion BST ("inion-larger" group), and with the ipsilateral BST ("lateral-larger" group). Repeated-measures analysis of variance (ANOVA) was conducted to test the effects of SITE (inion and ipsilateral BST, within-subjects factor) and GROUP (inion-larger and lateral-larger, between-subjects factor) on AMT. When the interaction was significant, AMT for one stimulation site in each of the group was compared using Student's *t* test as a *post hoc* analysis; that is, AMT for inion BST of the inion-larger group was compared with that of the lateral-larger group, and AMT for ipsilateral BST of the inion-larger group was compared with that of the lateral-larger group.

Onset MEP latencies were determined by one of the authors (RH): the shortest latency was measured from the superimposed waveforms by visual inspection (Chen et al., 2008). Coil position and subject name were blinded. To validate the reproducibility, another author (SO) conducted the same measurements and intraclass correlation coefficient (ICC) was calculated for the two sites (McGraw and Wong, 1996; Martin et al., 2009). We report ICC (A, 1) used by McGraw and Wong (1996) as an index of the absolute agreement of the measurements. The latencies were compared between the two sites of stimulation using the paired *t* test. With regard to the latency data from the 20 subjects (8 from inion-larger and 12 from lateral-larger group) with MEP amplitude adjustment, we studied the latency difference between the two stimulation sites within each subject. Namely, the latency difference (the onset latency of MEP to inion BST minus that to ipsilateral BST) was obtained. These values were compared with zero for each group using the one sample *t* test. In all analyses, a p value < 0.05 was considered as significant.

3. Results

3.1. MEP amplitudes

The mean ± SD of the stimulus intensity was 66.6 ± 14.5%MSO to obtain 1 mV MEP using inion BST. The box and whisker plot (Fig. 1C) demonstrates that MEP amplitudes to inion BST were distributed around 1 mV, but those to ipsilateral BST were larger. Paired *t* tests confirmed significantly larger MEPs to ipsilateral BST ($p = 0.005$). In 21 subjects (67.7%), the amplitude of MEP evoked by ipsilateral BST was larger than that by inion BST (Fig. 1A). Contralateral BST did not elicit clear MEPs, as shown in Fig. 2, in most subjects. Therefore, MEP using contralateral BST was not analyzed.

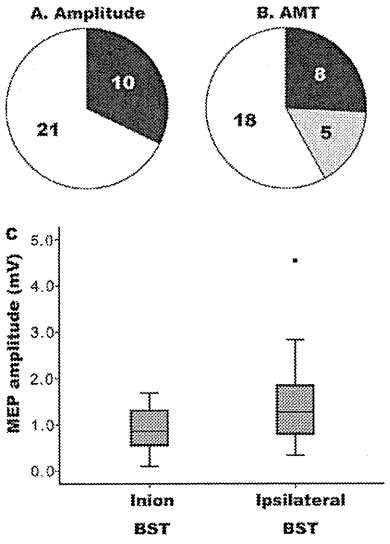

Fig. 1. Inter-individual variation in MEP amplitude and AMT. (A) Motor evoked potential (MEP) amplitude. Inion BST elicited larger MEPs in 10 subjects (black), while ipsilateral BST did in the other 21. (B) Active motor threshold (AMT). Eight subjects shown in black and 18 subjects in white had lower AMT, respectively, for inion BST and ipsilateral BST. The other five in gray had the same AMT for inion and ipsilateral BSTs. (C) Graph shows the distribution of MEP amplitudes for inion BST and ipsilateral BST. Boxes represent the range of the first quartiles and the third quartiles. The bars within the boxes are medians. The upper and lower ends of the whiskers show the maximum and minimum values except the upper end in ipsilateral BST, which indicates a 1.5 interquartile range from the median. An outlier in ipsilateral BST is depicted as a dot.

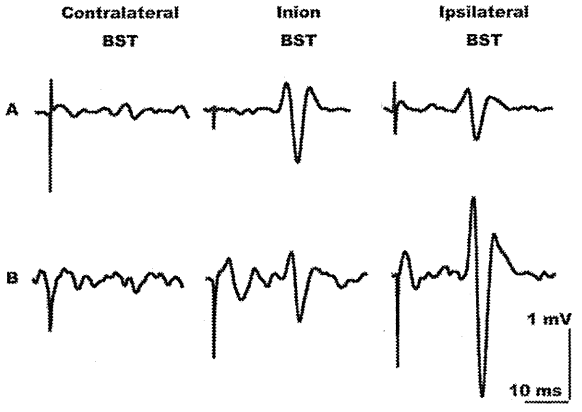

Fig. 2. Representative waveforms. Traces show averaged waveforms of MEP from two representative subjects (*A* and *B*) for the three stimulation points: inion BST, ipsilateral BST, and contralateral BST. In both subjects, inion BST elicited approximately 1 mV MEP in right FDI during muscle contraction. In the upper row (A), the MEP using ipsilateral BST is smaller than that using inion BST. In contrast, in the lower row (B), ipsilateral BST elicits much larger MEP than inion BST. Contralateral BST evoked no discernible MEP in either of the traces. Horizontal bar = 10 ms; vertical bar = 1 mV.

3.2. Active motor threshold

We found no significant difference in AMT between the inion and the ipsilateral BST when all subjects were analyzed together (57.3 ± 14.1%MSO for inion BST, and 55.3 ± 12.3%MSO for ipsilateral BST; $p = 0.16$). Fig. 1B shows that AMT for inion BST was lower in 8 subjects, and AMT for ipsilateral BST was lower in 18 subjects. The other five subjects showed equivalent AMT for the two stimulation sites. The MEP amplitude data and the AMT data were consistent. Subjects who had lower AMT for inion BST had larger MEP to inion BST than ipsilateral BST, and vice versa. Among the five subjects who showed equivalent AMT for inion and ipsilateral BST, two had larger MEP to the inion BST and three had larger MEP to the ipsilateral BST.

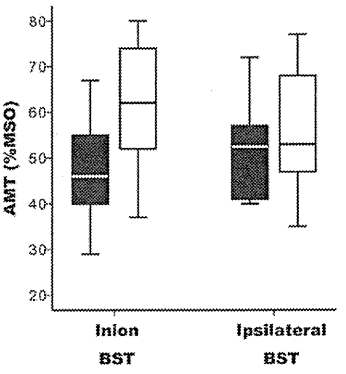

Fig. 3. Distributions of the active motor threshold (AMT). Distributions of AMT values are shown separately for different stimulation sites (inion BST and ipsilateral BST) and for different groups (inion-larger group, black; and lateral-larger group, white). The boxes show the range of the first and the third quartiles, and the white or black bars within the boxes show the medians. Ends of the whiskers represent the maximum and minimum values. The AMT for inion BST in the inion-larger group (black) is lower than the other three.

Repeated measures ANOVA on AMT revealed significant interaction between GROUP and SITE ($F(1, 30) = 33.1$; $p < 0.001$). Quite interestingly, inspection of the distribution of the actual AMT values (Fig. 3) suggests that this interaction occurred largely because AMT was lower for inion BST in the inion-larger group than in the lateral-larger group. This notion was confirmed using *post hoc* analysis showing significant difference in AMT for inion BST between the groups (mean ± SD; 47.2 ± 11.6%MSO for inion-larger group and 62.1 ± 12.7%MSO for lateral-larger group, $p = 0.004$). In contrast, the two groups were similar in AMT for ipsilateral BST (mean ± SD; 53.0 ± 11.2%MSO for inion-larger group and 56.4 ± 12.9%MSO for the lateral-larger group, $p = 0.48$).

3.3. Latency

Onset latency was 17.6 ± 1.30 (mean ± SD) ms for inion BST and 17.3 ± 1.35 ms for ipsilateral BST; these were significantly different (paired *t* test, $p = 0.03$). ICC (A, 1) was 0.941 ($p < 0.001$) for inion BST and 0.914 ($p < 0.001$) for ipsilateral BST. To investigate the latency difference further, MEP amplitude was adjusted to the same level in 20 subjects (the inion-larger group ($n = 8$), 0.96 ± 0.47 mV for inion BST and 0.92 ± 0.40 mV for ipsilateral BST ($p = 0.798$); the lateral-larger group ($n = 12$), 0.97 ± 0.50 mV for inion BST and 1.00 ± 0.42 mV for ipsilateral BST ($p = 0.718$)). In the inion-larger group, the latency difference did not differ significantly from zero ($p = 0.460$). However, significant difference was found in the lateral-larger group ($p = 0.013$). Latencies for the ipsilateral BST were significantly shorter than those for the inion BST in the lateral-larger group (Fig. 4A). Spinal conduction time was longer than 3 ms in all the subjects (Fig. 4B), which excludes the possibility that spinal nerve root was directly stimulated by ipsilateral BST.

4. Discussion

We demonstrated that about two-thirds of the subjects had larger MEPs to the ipsilateral BST than the inion BST. Furthermore, the amplitude data were consistent with the AMT data. Subjects whose

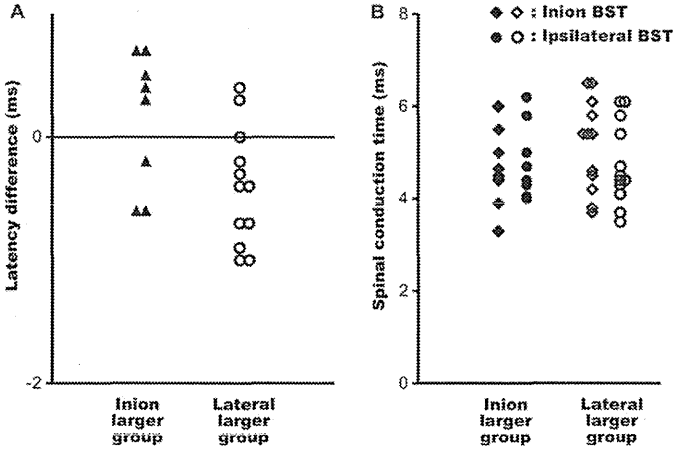

Fig. 4. Latency difference and spinal conduction time. (A) Latency difference between the inion and ipsilateral BSTs in each group (triangles for the inion-larger group, and circles for the lateral-larger group). The ordinate shows the difference in the two latencies: negative values mean that latency for ipsilateral BST is shorter than that for inion BST. In the lateral-larger group, ipsilateral BST shows the shorter latencies. (B) Spinal conduction time, which is the difference between the BST latency and spinal root stimulation latency, is plotted for each subject. Mean ± standard deviation of the spinal conduction time was 4.31 ± 0.65 ms for inion BST and 4.46 ± 0.72 ms for ipsilateral BST in the inion-larger group; 4.77 ± 0.67 ms for inion BST and 4.29 ± 0.60 ms for ipsilateral BST in the lateral-larger group.

MEPs to ipsilateral BST were larger than those using inion BST had lower or same AMT for ipsilateral BST than that for inion BST, and vice versa. Contralateral BST was considered to be less useful from clinical point of view, because it did not elicit clear MEPs in most of the subjects. A novel point of this study is that significant inter-individual difference in terms of the stimulation site for BST was found based on a relatively large number of subjects, offering practical benefit for clinical and research use of BST.

It is sometimes difficult to obtain reliable MEPs to BST in some subjects with neurological disorders. Therefore, we developed double pulse BST to overcome this difficulty (Matsumoto et al., 2008). Although this procedure is tolerable and useful, it is more desirable to perform BST with lower stimulus intensity and smaller number of total pulses. Based on our present findings, it might be practically preferred to perform ipsilateral BST first for subject's comfort.

Our results will also help to refine the procedure of CBI. Previous studies of CBI have set the conditioning stimulus intensity just below AMT of inion BST for cerebellar stimulation (Daskalakis et al., 2004; Pinto and Chen, 2001; Ugawa et al., 1995). However, a recent study demonstrated that stimuli using this intensity can still activate the pyramidal tract, underscoring the need for caution in determining stimulus intensity for cerebellar stimulation (Fisher et al., 2009). Indeed, pyramidal tract activation can be a major confounding factor of cerebellar stimulation (Meyer et al., 1994; Ugawa, 2009; Ugawa et al., 1995). For that reason, it would be preferable to use the stimulus intensity below AMT. Our results demonstrated that nearly 60% (18/31) of subjects had lower AMT for ipsilateral BST. In these subjects, it is possible that concomitant pyramidal tract activation strongly affects the results of CBI if the conditioning stimulus intensity is determined in relation to AMT for inion BST. Therefore, it would be reasonable to obtain both AMTs for inion and ipsilateral BST to avoid confounding pyramidal tract activation when testing CBI.

What caused this inter-individual difference? First, direct stimulation of the spinal motor root by ipsilateral BST might be one of the possibilities considering the fact that the coil goes closer to the roots in the ipsilateral BST; however, we consider this unlikely, because the spinal conduction times in Fig. 4B are longer than 3 ms in all the subjects, which is in line with previous reports (e.g. Ugawa et al., 1994) and excludes the possibility that ipsilateral BST directly activated motor roots.

At least two other possibilities should be discussed. One is that the activation site by inion and ipsilateral BST might be different in the brainstem because of possible differences in the distribution of the induced eddy currents among subjects. Then, the threshold for each activation site can be expected to vary among individuals. The other is, if the activation site is the same for both (i.e. inion and ipsilateral) BSTs, then the eddy current size at that activation site can be different for each stimulation site among subjects, possibly because of the difference in skull shape or in the composition of the tissue between the coil and the activation site. The latency difference shown in Fig. 4A might favor the notion of different activation sites between different coil positions: ipsilateral BST might activate more distal point of the pyramidal tract in the lateral-larger group. On the other hand, in the inion-larger group, the significantly lower AMT for inion BST and similar latencies for inion and ipsilateral BSTs might indicate that the activation site is the same for the two BSTs and that the efficacy difference is attributable to the different amounts of eddy currents between two stimulations at the same activation site. Because currents induced by TMS attenuates with increasing distance from the coil, the findings that contralateral BST did not evoke large MEPs in most subjects lead us to infer that one plausible candidate for the activation sites is a point distal to the decussation, i.e. ipsilateral to the recording site, as described in a previous report (Ugawa et al., 1994). However, in the present study, MEP latencies were measured in the contracted muscle because of the difficulty in obtaining stable MEPs at rest. This might have increased the measurement errors. Although high ICC (A, 1) values suggest good agreement of the measurements, we should be quite cautious to draw a firm conclusion about the site of activation on the basis of the small latency difference of 0.3 ms on average. Therefore, further research is necessary to determine the precise activation site.

One limitation of this study might be that only three stimulation points were tested. However, it is often difficult to perform BSTs using high intensities repeatedly to map the optimal stimulation site precisely. We aimed at proposing an easier and better way to perform BST; the difference between inion and ipsilateral BST among subjects described in this report fits this purpose. Secondly, our results are partially inconsistent with those presented in our previous report (Ugawa et al., 1994), which showed that the AMT was lowest at the inion. The small number of subjects studied in

a previous report might lead this inconsistency. Finally, we did not examine left hand muscles and leg muscles; therefore, further research is warranted as to in what proportion of subjects ipsilateral BST is more effective than inion BST in those muscles.

In conclusion, we demonstrated that inion and ipsilateral BST have different efficiencies for eliciting MEPs in different subjects. We can reduce the intensity and the total pulse number needed to perform BST using the method described here. The results are possibly beneficial to the procedure of CBI.

Conflict of Interest

None declared.

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RESEARCH ARTICLE

Short-interval intracortical inhibition in Parkinson's disease using anterior-posterior directed currents

R. Hanajima · Y. Terao · Y. Shirota · S. Ohminami · S. Nakatani-Enomoto · S. Okabe · H. Matsumoto · R. Tsutsumi · Y. Ugawa

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Abstract Reduced short-interval intracortical inhibition (SICI) is reported in Parkinson's disease (PD) and is considered to reflect abnormal GABAergic inhibitory system of the primary motor cortex in PD. We have recently shown, however, that SICI using anterior-posterior directed currents in the brain was normal in focal dystonia even though that using posterior-anterior currents was abnormal, indicating that the GABAergic system of the primary motor cortex is largely normal in dystonia. Here, we studied SICI in PD to clarify whether the GABAergic system is completely impaired in PD. We used paired-pulse transcranial magnetic stimulation to study SICI at interstimulus intervals of 3 and 4 ms with anterior-posterior or posterior-anterior directed currents in eight PD patients and ten healthy volunteers. The amount of SICI with posterior-anterior directed currents was reduced in PD patients compared with healthy volunteers; in contrast, SICI studied with anterior-posterior directed currents was normal in PD patients. These observations may be due to the difference in I-wave composition generated by the two directed currents and/or the difference in responsible inhibitory interneurons for the inhibition between the two current directions. We suggest that some or a part of inhibitory interneurons are not involved in PD. This discrepancy between SICI using posterior-anterior and anterior-posterior directed currents experiments may provide additional information about the circuits of the motor cortex.

Keywords Transcranial magnetic stimulation · GABAergic inhibition · I-waves · Parkinson disease · Short-interval intracortical inhibition

R. Hanajima (✉) · Y. Terao · Y. Shirota · S. Ohminami · S. Nakatani-Enomoto · S. Okabe · H. Matsumoto · R. Tsutsumi · Y. Ugawa
Department of Neurology, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: hanajima-tky@umin.ac.jp

S. Nakatani-Enomoto · Y. Ugawa
Department of Neurology, Fukushima Medical University, Fukushima, Fukushima, Japan

Introduction

Many investigations have examined the changes in the internal circuits of the primary motor cortex (M1) in Parkinson's disease (PD). Paired-pulse transcranial magnetic stimulation (TMS; Kujirai et al. 1993) was used to demonstrate a reduction in short-interval intracortical inhibition (SICI) in PD under the off condition (Ridding et al. 1995a, b; Hanajima et al. 1996; Ziemann et al. 1996; Strafella et al. 2000; Bares et al. 2003), an abnormality that was normalized by L-DOPA (Ridding et al. 1995a, b). The straightforward interpretation of this SICI reduction is that the gamma-amino butyric acid (GABA)-A mediated inhibitory system of M1 is involved in PD due to basal ganglia-motor cortical circuit dysfunction induced by dopamine deficiency. On the other hand, several studies reported normal SICI in PD (Berardelli et al. 1996; Hanajima et al. 1996; MacKinnon et al. 2005; Chu et al. 2009). This discrepancy is probably caused by different disease stages of the studied patients (Hanajima et al. 1996; MacKinnon et al. 2005), different states of muscle contraction (active or resting) during the experiment (Berardelli et al. 1996), or different conditioning stimulus (CS) intensities (MacKinnon et al. 2005; Chu et al. 2009). MacKinnon et al. (2005) proposed that intracortical facilitation overlapped SICI by strong CS. Taken together, these observations suggest that reduced SICI is caused by

several combined mechanisms in addition to GABA-A-mediated inhibitory system involvement in PD, although controversy still remains regarding SICI in PD.

The discrepancy in SICI in PD may be resolved by controlling the way motor-evoked potentials (MEPs) are induced for studying SICI; the amount of SICI is influenced by the relative amounts of early (I1) and late (I3) I-waves in the investigation; I3 waves are more subject to SICI than I1 waves, supporting the idea that I3-wave suppression is an effect of GABA-A-mediated inhibition within the motor cortex (Nakamura et al. 1997; Hanajima et al. 1998). We recently demonstrated that SICI studied with anterior-posterior (AP) directed currents is normal in focal dystonia, even though SICI with posterior-anterior (PA) directed currents is abnormal (Hanajima et al. 2008). AP currents preferentially activate I3 waves, while conventional PA currents activate I1 waves (Sakai et al. 1997). Accordingly, responses generated by AP currents may be more sensitive to SICI than those generated by PA currents. Based on the observed discrepancy between SICIs using AP and PA currents (Hanajima et al. 2008), we concluded that the GABA-A-mediated system of M1 is largely normal in dystonia. We therefore designed the current investigation to examine SICI using AP-induced currents in PD.

Subjects and methods

Eight patients with PD (44–77 years old, five men and three women; Table 1) and ten age-matched healthy volunteers (43–73 years old, six men and four women) were included in the present study. All PD patients had idiopathic PD according to the British Parkinson's Disease Society Brain Bank criteria (Daniel and Lees 1993). Four PD patients were studied de novo before starting medication, and the other four patients were studied under the off condition. No anti-Parkinsonian drugs were taken at least 12 h before the experiment. The PD patients presented with a mean unified Parkinson's disease rating scale (UPDRS) III (Fahn and Elton 1987) score of 18.6 (range 4–24; Table 1).

SICI was studied by paired-pulse TMS (Kujirai et al. 1993). MEPs were recorded from the first dorsal interosseous muscle of the side more affected by PD symptoms in the relaxed condition, amplified, and filtered at 100 and 3 kHz (Biotop; GE Marquette Medical Systems Japan Inc., Japan). We set the low-cut filter relatively higher than usual to remove the stimulus artifact; the sizes of the MEPs subsequently became smaller, but the ratio of conditioned MEP to test MEP did not differ from the ratio of MEPs recorded with the conventional low-cut filter. These filters, therefore, did not impact our main observations. CS and test stimuli were administered through the same figure 8-shaped coil connected to a Bistim module linked with two Magstim 200 magnetic stimulators (The Magstim Company Ltd., UK). The coil was placed at the motor point for the first dorsal interosseous muscle and oriented anteriorly or posteriorly parallel with the sagittal plane to induce PA- or AP-directed currents in the brain, respectively. We did not use a current direction of 45° inward from the mid-sagittal line because currents in this direction readily induce D-waves in some subjects, which may serve as another confounding factor when interpreting SICI results (Sakai et al. 1997; Di Lazzaro et al. 2004).

The active motor threshold (AMT) of each directed current was calculated as the intensity that induced an MEP of 100 μv in 5 out of 10 trials. The intensity of the CS was set at 90% AMT, and the test stimuli were set to induce approximately 0.5 mV MEP for each direction. The interstimulus intervals (ISIs) were 3 and 4 ms. We did not use ISIs of 1 or 2 ms because the inhibition at these two intervals must be generated by mechanisms other than GABAergic inhibition (Fisher et al. 2002; Hanajima et al. 2003). We collected 10 MEPs for each ISI and 15 MEPs for the control condition in which the test stimulus was given alone and we calculated the ratio of the mean amplitude of conditioned responses to that of control responses (size ratio) at each ISI.

Table 1 Patient data

Patient No.	Gender	Age	Condition	L-DOPA equivalent dose (mg/day)	Disease duration (years)	UPDRS III
1	M	44	De novo	0	2	4
2	W	75	De novo	0	2	12
3	M	69	De novo	0	7	20
4	W	58	De novo	0	1	22
5	M	64	Off	1,090	23	21
6	W	76	Off	200	1	23
7	M	59	Off	775	11	23
8	M	77	Off	650	9	24

Statistical analyses were performed with SPSS version 14 (Japan IBM Inc., Tokyo). For comparisons of SICI between PD patients and healthy volunteers, we analyzed the effects of subject group (GROUP: PD patients and healthy volunteers) and ISI (3 and 4 ms) on the size ratios of the MEPs using two-factorial repeated measure analysis of variance for each direction (PA-directed currents or AP-directed currents). Paired t test was used in post hoc analyses when a significant effect was found for ISI.

Correlations between SICI and UPDRS III score, disease duration, and daily Dopa equivalent dose (DED) were studied with linear regression analyses. SICI was represented by the average size ratio at ISIs of 3 and 4 ms in this analysis. P-values less than 0.05 were considered significant.

Results

The mean AMT with PA-directed currents was 37.7 ± 9.8 (± standard deviation, SD) percent maximum stimulator output (%MSO) for healthy volunteers and 42.7 ± 5.5%MSO for PD patients. The mean AMT with AP-directed currents was 50.8 ± 8.8%MSO for healthy volunteers and 63.1 ± 15.2%MSO for PD patients. The mean AMT was thus significantly higher with AP-directed currents than PA-directed currents (paired t test $P < 0.01$ for both groups). There were no significant differences in AMT between the healthy group and the PD group. The mean test intensity with PA-directed currents was 71.1 ± 18.1%MSO in healthy volunteers and 67.3 ± 15.3%MSO in PD patients, and that with AP-directed currents was 77.6 ± 18.6%MSO in healthy volunteers and 81.8 ± 10.0%MSO in PD patients. These intensities did not differ significantly between the two subject groups. The mean test MEP amplitude induced by PA-directed currents was 0.47 ± 0.09 (± standard error, SE) mV in healthy volunteers and 0.47 ± 0.09 mV in PD patients, and that by AP-directed currents was 0.42 ± 0.05 mV in healthy volunteers and 0.49 ± 0.07 mV in PD patients. The latency of test MEP by PA-directed currents was 22.0 ± 0.7 (± SD) ms in healthy volunteers and 22.0 ± 1.0 ms in PD patients, and that by AP-directed currents was 22.9 ± 0.5 ms in healthy volunteers and 23.6 ± 0.7 ms in PD patients. The MEP latency by AP-directed currents was significantly longer than that by PA-directed currents (paired t test $P < 0.01$), but we detected no significant differences between the two subject groups.

Figure 1a depicts the mean (± SE) amplitude ratios in healthy volunteers (ISI 3 ms, 0.45 ± 0.07; ISI 4 ms, 0.63 ± 0.13) and in PD patients (ISI 3 ms, 0.77 ± 0.10; ISI 4 ms, 1.01 ± 0.12) using PA-directed currents. Two-factorial repeated measure analysis of variance [ISI and GROUP (PD patients and healthy volunteers)] revealed significant effects of ISI [F (1; 16) = 6.03, $P < 0.05$] and GROUP [F (1; 16) = 7.15, $P < 0.05$], but no interaction between ISI and GROUP. The ratio at an ISI of 3 ms was significantly less than that at an ISI of 4 ms (paired t test, $P < 0.05$), and the ratio was significantly higher in PD patients than in healthy group.

With AP-directed currents (Fig. 1b), the mean (± SE) amplitude ratios were 0.16 ± 0.04 at an ISI of 3 ms and 0.32 ± 0.08 at an ISI of 4 ms in healthy volunteers; for PD patients the respective values were 0.17 ± 0.06 and 0.32 ± 0.08. The ISI [F (1; 13) = 8.96, $P < 0.05$] significantly affected the amplitude ratio. The GROUP did not significantly affect the ratio, and there was no significant interaction between ISI and GROUP.

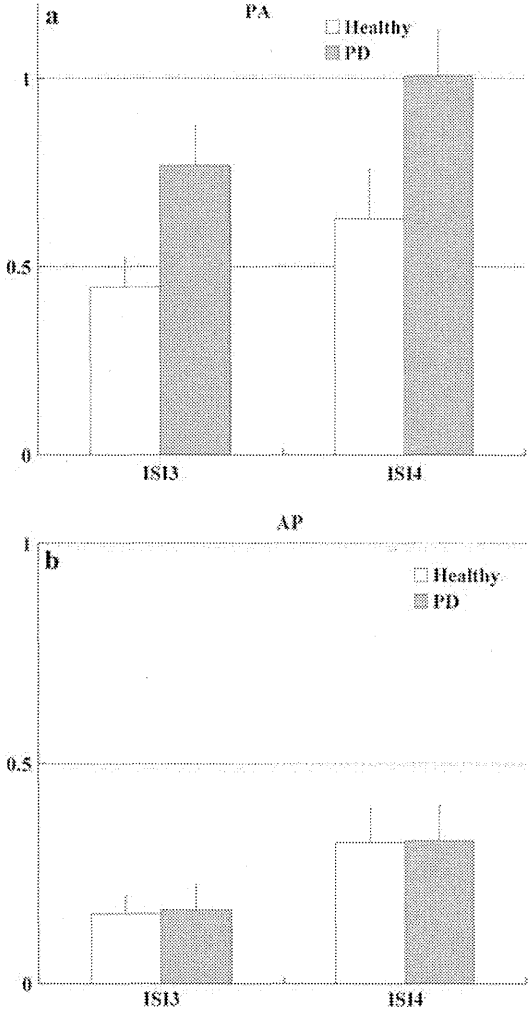

Fig. 1 SICI in PD patients (*gray bars*) and healthy volunteers (*white bars*). **a** Mean (±SE) size ratios obtained using PA-directed currents at ISI of 3 or 4 ms. **b** Mean size ratios obtained using AP-directed currents