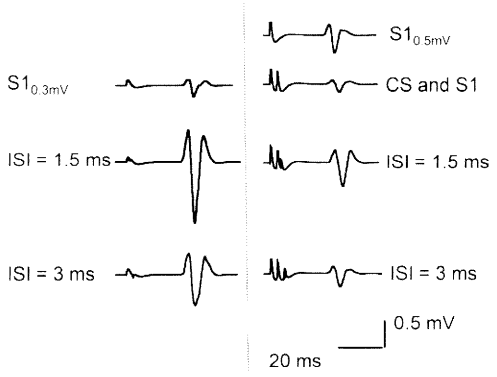

FIG. 5. Representative responses from 1 participant. Traces show averaged responses from 1 participant. *Left*: the SICF using $S1_{0.3mV}$ and S2 of 140% AMT. At both the 1st peak (ISI = 1.5 ms) and the 2nd peak (ISI = 3 ms), the MEP sizes are much larger than that of S1 alone ($S1_{0.3mV}$). *Right*: the results of triple-pulse stimulation using CS of 120% AMT, $S1_{0.5mV}$, and S2 of 140% AMT in *experiment 3*. The 1st peak (ISI = 1.5 ms) is fairly facilitated compared with SICI alone (CS and S1), but the 2nd peak (ISI = 3 ms) is considerably small in the presence of CS.

peaks of SICF were differently modulated by the preceding CS and that our findings might be produced by some I-waves interaction in each SICF peak.

We reported earlier that the first peak of SICF consists of the summation of S1 and S2 applied 1.5 ms later along the I2 wave pathway (Hanajima et al. 2002). The S1 activates interneurons for I2 waves, which then deliver subliminal depolarization of interneurons along its pathway 1.5 ms later. The following S2 activates these subliminally depolarized interneurons when the S2 is applied at a preferred time (i.e., 1.5 ms later than the S1). This activation produces additional I2 waves, and leads to the facilitation occurring at I2 latency. Similarly, the second peak of SICF is probably generated at I3 latency from S1 in the following way: the S1 activates interneurons for I3 waves, which subliminally depolarize interneurons along its pathway 3 ms later; then S2 directly activates these interneurons. On the other hand, I3 waves are more suppressed by CS in the paired-pulse paradigm than I1 or I2 waves (Di Lazzaro et al. 1998b; Hanajima et al. 1998). Considering these two arguments, our results are explained as follows: in the presence of CS, S1 produces less activation of interneurons for I3 waves, which in turn engenders less subliminal depolarization of interneurons along its pathway 3 ms later. Consequently, S2 coming 3 ms later cannot activate additional interneurons sufficiently; then the second peak of SICF does not emerge.

Furthermore, this suppression was similarly significant when CS was applied at −5 ms, although SICI itself was not evoked at an ISI of −5 ms, in line with previous findings (Hanajima et al. 2007). In fact, SICI is considered to be produced by GABAergic inhibitory postsynaptic potential (IPSP) elicited by CS (Kujirai et al. 1993; Ziemann et al. 1996a). The IPSPs last more than 10 ms (McCormick 1989; Williams and Stuart 2003). We already demonstrated that I3 waves were inhibited at an ISI of 10 ms (Hanajima et al. 1998). The IPSP produced by CS would be partly effective at 5 ms later, which might explain SICF suppression by CS at an ISI of −5 ms.

In contrast to the second peak of SICF, why was the other peak less susceptible to CS? The first peak of SICF is believed to result from the interaction between I1 and I2 waves, both of which are less suppressed by CS than later I waves in the paired-pulse paradigm (SICI). In this context, the smaller susceptibility of I2 waves to SICI might simply explain less modulation of the first peak in the presence of CS. As for the third peak, which is considered to be mediated by another interaction of I waves, we did not find a significant peak in *experiment 2*. A previous study demonstrated that 90% RMT S2 did not produce the third peak but that 100% RMT S2 did (Chen and Garg 2000). The present results agree with this study because our 140% AMT, that is the intensity of the higher S2, corresponded to ~90–95% RMT as calculated by Tables 1 and 2. Stimulus intensity dependency of the third peak has remained to be studied.

Effects of CS intensity on SICF

In the present study, CS intensity had considerable influence on SICF: CS below or around AMT disinhibited, albeit non-significantly, the first and second peak of SICF, whereas CS above AMT significantly suppressed the second peak of SICF and tended to suppress the first peak of SICF, too. The results bear out the importance of CS intensity in the triple-pulse paradigm. We favor the view that the suppression of the second

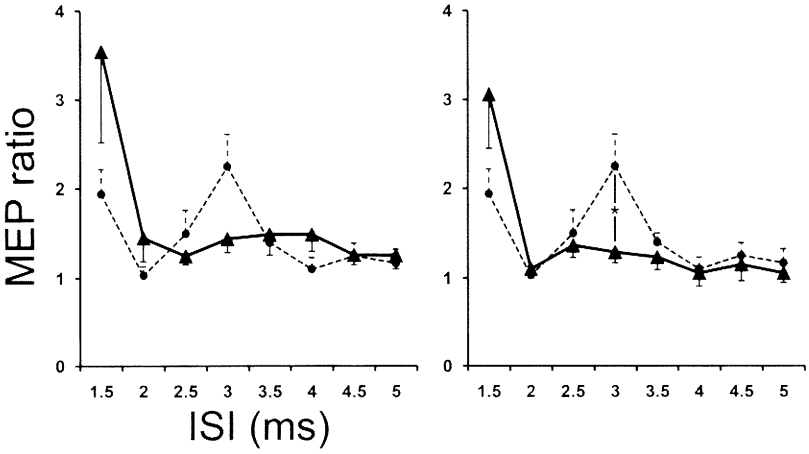

FIG. 6. Mean time courses of triple-pulse stimulation at CS-S1 ISI of −5 ms. ▲—▲, triple-pulse stimulation, in which a CS-S1 ISI of −5 ms was used. The 2nd peak of the SICF is suppressed in the sessions using CS of 100% AMT (*A*) and 120% AMT (*B*). ●- - -●, SICF alone using $S1_{0.5mV}$ with S2 of 140% AMT. Error bars represent SE. *, statistical significance ($P < 0.05$).

peak of SICF is ascribed to the direct effect of inhibitory interneurons on I3 wave and that the "disinhibition" of SICF peaks by lower CS intensity is mediated by different groups of inhibitory interneurons (Wagle-Shukla et al. 2009). In this context, there may be at least three explanations to account for the stimulus intensity dependency of the modulation of SICF. One possibility is that the cortical neurons stimulated by CS are different depending on the stimulation intensity. CS with lower intensity could activate interneurons responsible for disinhibition more preferentially and higher CS elicits firing of interneurons that mediate SICI, leading to inhibition of I waves. Furthermore, considering the fact that disinhibition was observed only when CS intensity was low, activation of the interneurons mediating disinhibition may show a ceiling effect with higher stimuli. Another possibility relates to a contamination of SICF elicited by higher intensity CS (Peurala et al. 2008). It is possible that the contamination of SICF with a preceding CS results in refractoriness of neurons responsible for SICF, leading to the suppression of SICF peaks. This possibility is, however, less likely in view of the argument of Peurala et al. (2008) that it should be necessary for the CS to be >90% RMT to elicit a contaminating SICF effect because the CS needs to evoke many I waves enough for succeeding temporal summation (Peurala et al. 2008). Here our highest intensity of CS (i.e., 120% AMT) was approximately equal to 80% RMT as calculated by Tables 1 and 2, so that the CS is insufficient to evoke a huge SICF effect as the S1 in the SICF paradigm. Furthermore, CS intensity of 100% AMT, less contaminated by SICF (Peurala et al. 2008), tended to suppress the second peak of SICF using 140% AMT S2, although the effect was not statistically significant. Thus the contamination of SICF alone does not simply account for the suppression of the second peak of SICF by CS. Nevertheless future studies are needed to determine the effect of the contamination of SICF in the triple-pulse paradigm. Finally, variation in S2 intensity might be partly responsible for the present findings. Because the significant suppression of the second peak of SICF was observed in the condition using the higher intensity S2 (140% AMT), we cannot exclude the possibility that the parameters of the present study evoked much stronger SICF than in the previous study (Wagle-Shukla et al. 2009) and favored findings of inhibition of SICF, similar to a ceiling effect.

Triple-pulse paradigm in comparison with paired-pulse paradigm

It is particularly interesting that a clear suppression of the second peak of SICF was the most prominent with the CS of 120% AMT. Triple-pulse stimulation using 100% AMT CS and 140% AMT S2 showed a tendency for suppression of the second peak (Fig. 4*E*, *experiment 3*), but it was not statistically significant. By contrast, the most effective suppression was found at 100% AMT using the paired-pulse paradigm (Fig. 2, *experiment 1*); in fact, we found a U-shaped curve of SICI in *experiment 1*, compatible with previous studies (Orth et al. 2003; Ziemann et al. 1996b). Consequently, an apparent discrepancy exists between the paired- and triple-pulse paradigms. Recent reports described that SICI was contaminated by SICF at higher CS intensity above AMT and that only a net inhibition could be observed at these conditioning intensities (Ortu et al. 2008; Peurala et al. 2008). Importantly, these recent studies attributed the cause of the U-shaped curve or the lack of suppression by higher CS intensity mainly to contamination of SICF, not to reduction in SICI. Thus it is possible that inhibitory effects of CS are identical or may be stronger when a higher CS is used. In fact, IPSP is increased with higher stimulation intensity (Williams and Stuart 2003). Besides, one previous study has shown that inhibitory effect of a higher CS on I3 wave is stronger when I3 wave was elicited preferentially (Hanajima et al. 1998). On the basis of these arguments, our present results from triple-pulse stimulation might be interpreted as follows; a higher CS produces more inhibitory effect, but it is blurred in the usual paired-pulse paradigm testing SICI because of the contamination of SICF. On the other hand, the triple-pulse paradigm performed in the present study could possibly reveal an inhibitory effect of CS in its stronger end by demonstrating the suppression of SICF.

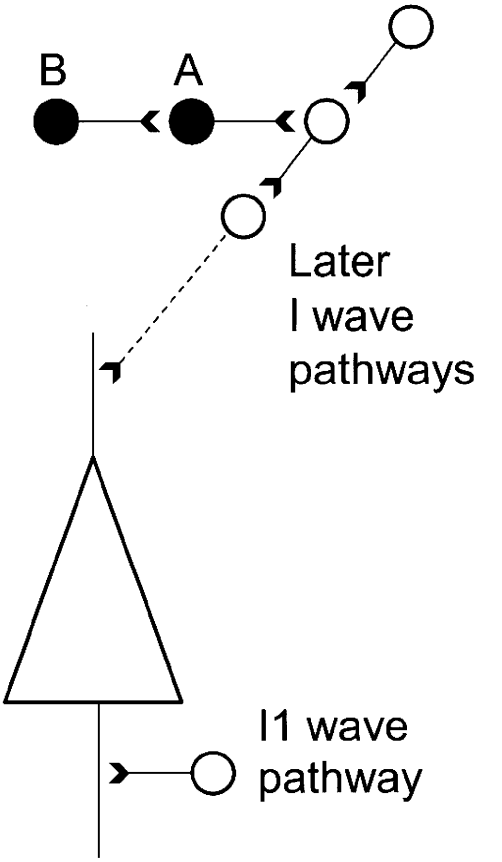

FIG. 7. A proposed hypothesis. A hypothesis to explain the present results are depicted schematically. △, a corticospinal neuron; ○ and ●, interneurons. A chain of facilitatory interneurons (○) produce later I waves when a single pulse transcranial magnetic stimulation (TMS) is applied. Inhibitory interneurons (● A) mainly synapsing with interneurons for I3 wave are activated when a preceding CS was applied, mediating SICI. On the other hand, SICF occurs when S2 follows S1, by temporal summation. When a CS with a higher intensity is used, it also activates some of the facilitatory interneurons, and SICI is contaminated by SICF. We propose another group of inhibitory interneurons (● B), which have lower threshold than interneurons A, to account for the present findings. In the triple-pulse paradigm, a lower intensity CS could possibly activate interneurons B predominantly, leading to facilitation of SICF by inhibiting interneurons A, which finally dis-inhibits I3 waves production. On the other hand, a strong CS can preferentially activate interneurons A than B, resulting in the suppression of the second peak of SICF through inhibition of I3 waves.

Technical consideration

Because we have conducted the experiments over several days, some other factors than intracortical process might have affected the present results, such as fatigue or intraindividual variability. On the other hand, however, it was also necessary to divide the experiments into several portions since too long experiments would also have made the subjects fatigued.

Proposed hypotheses and conclusion

On the basis of the discussion in the preceding text, CS can modulate peaks of SICF differently, and this modulation might be dependent on stimulus intensity. CS below or around AMT might disinhibit the first and the second peaks and CS above AMT can inhibit the second, and possibly the first, peaks. We have shown that stimulus intensity had much influence on the effect of CS on SICF. We favor a model that some inhibitory interneurons send inputs to other interneurons that mediate SICI, which itself is similar to the model proposed by Wagle-Shukla et al. (2009). This hypothesis is shown schematically in Fig. 7. The former inhibitory interneurons (neuron B in Fig. 7) had a lower threshold for TMS and exert disinhibition by inhibiting the latter inhibitory interneurons (neuron A in Fig. 7). These two groups of interneurons mainly affect I3 waves. We depicted a chain of interneurons in Fig. 7, but it is also possible that different I waves are produced by different groups of interneurons because the mechanism of I wave generation is unknown. In that model, neurons A and B described in the preceding text might predominantly influence interneurons that mediate I3 wave in the same way. The effects of SICI and SICF converge mainly on the production of I3 waves.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

Amassian VE, Stewart M, Quirk GJ, Rosenthal JL. Physiological basis of motor effects of a transient stimulus to cerebral cortex. *Neurosurgery* 20: 74–93, 1987.

Chen R, Garg R. Facilitatory I wave interaction in proximal arm and lower limb muscle representations of the human motor cortex. *J Neurophysiol* 83: 1426–1434, 2000.

Day BL, Dressler D, de Noordhout AM, Marsden CD, Nakashima K, Rothwell JC, Thompson PD. Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. *J Physiol* 412: 449–473, 1989.

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali P, Rothwell JC. Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. *Electroenceph Clin Neurophysiol* 109: 397–401, 1998a.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC. Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. *Exp Brain Res* 119: 265–268, 1998b.

Fisher RJ, Nakamura Y, Bestmann S, Rothwell JC, Bostock H. Two phases of intracortical inhibition revealed by transcranial magnetic threshold tracking. *Exp Brain Res* 143: 240–248, 2002.

Hamada M, Hanajima R, Terao Y, Arai N, Furubayashi T, Inomata-Terada S, Yugeta A, Matsumoto H, Shirota Y, Ugawa Y. Quadro-pulse stimulation is more effective than paired-pulse stimulation for plasticity induction of the human motor cortex. *Clin Neurophysiol* 118: 2672–2682, 2007.

Hamada M, Terao Y, Hanajima R, Shirota Y, Nakatani-Enomoto S, Furubayashi T, Matsumoto H, Ugawa Y. Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. *J Physiol* 586: 3927–3947, 2008.

Hanajima R, Furubayashi T, Iwata NK, Shiio Y, Okabe S, Kanazawa I, Ugawa Y. Further evidence to support different mechanisms underlying intracortical inhibition of the motor cortex. *Exp Brain Res* 151: 427–434, 2003.

Hanajima R, Nomura Y, Segawa M, Ugawa Y. Intracortical inhibition of the motor cortex in Segawa disease (DYT5). *Neurology* 68: 1039–1044, 2007.

Hanajima R, Ugawa Y, Terao Y, Enomoto H, Shiio Y, Mochizuki H, Furubayashi T, Uesugi H, Iwata NK, Kanazawa I. Mechanisms of intracortical I-wave facilitation elicited with paired-pulse magnetic stimulation in humans. *J Physiol* 538: 253–261, 2002.

Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, Kanazawa I. Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. *J Physiol* 509: 607–618, 1998.

Ilic TV, Meintzschel F, Cleff U, Ruge D, Kessler KR, Ziemann U. Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity. *J Physiol* 545: 153–167, 2002.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD. Corticocortical inhibition in human motor cortex. *J Physiol* 471: 501–519, 1993.

MacKinnon CD, Gilley EA, Weis-McNulty A, Simuni T. Pathways mediating abnormal intracortical inhibition in Parkinson's disease. *Ann Neurol* 58: 516–524, 2005.

McCormick DA. GABA as an inhibitory neurotransmitter in human cerebral cortex. *J Neurophysiol* 62: 1018–1027, 1989.

Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H. Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. *J Physiol* 498: 817–823, 1997.

Orth M, Snijders AH, Rothwell JC. The variability of intracortical inhibition and facilitation. *Clin Neurophysiol* 114: 2362–2369, 2003.

Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC. Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. *J Physiol* 586: 5147–5159, 2008.

Patton HD, Amassian VE. Single- and multiple-unit analysis of cortical stage of pyramidal tract activation. *J Neurophysiol* 17: 345–363, 1954.

Peurala SH, Müller-Dahlhaus JFM, Arai N, Ziemann U. Interference of short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF). *Clin Neurophysiol* 119: 2291–2297, 2008.

Roshan L, Paradiso GO, Chen R. Two phases of short-interval intracortical inhibition. *Exp Brain Res* 151: 330–337, 2003.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A. The Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* 120: 2008–2039, 2009.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevid MR, Hallett M, Katayama Y, Lücking CH, Maertens de Noordhout AL, Marsden CD, Murray NMF, Rothwell JC, Swash M, Tomberg C. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroenceph Clin Neurophysiol* 91: 79–92, 1994.

Sakai K, Ugawa Y, Terao Y, Hanajima R, Furubayashi T, Kanazawa I. Preferential activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil. *Exp Brain Res* 113: 24–32, 1997.

Tokimura H, Ridding MC, Tokimura Y, Amassian VE, Rothwell JC. Short latency facilitation between pairs of threshold magnetic stimuli applied to human motor cortex. *Electroenceph Clin Neurophysiol* 101: 263–272, 1996.

Wagle-Shukla AZ, Ni Z, Gunraj CA, Bahl N, Chen R. Interactions between short interval intracortical facilitation and short interval intracortical inhibition and intracortical facilitation in human primary motor cortex. *J Physiol* 587: 5565–5678, 2009.

Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. *Electroenceph Clin Neurophysiol* 108: 1–16, 1998.

Williams SR, Stuart GJ. Voltage- and site-dependent control of the somatic impact of dendritic IPSPs. *J Neurosci* 23: 7358–7367, 2003.

Ziemann U, Lönecker S, Steinhoff BJ, Paulus W. The effect of lorazepam on the motor cortical excitability in man. *Exp Brain Res* 109: 127–135, 1996a.

Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. *J Physiol* 496: 873–881, 1996b.

Ziemann U, Tergau F, Wassermann EM, Wischer S, Hildebrandt J, Paulus W. Demonstration of facilitatory I wave interaction in the human motor cortex by paired transcranial magnetic stimulation. *J Physiol* 511: 181–190, 1998.

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Efferent and afferent evoked potentials in patients with adrenomyeloneuropathy

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ABSTRACT

Objective: This paper investigates efferent and afferent conductions of the central nervous system by various evoked potentials in patients with adrenomyeloneuropathy (AMN).
Patients and methods: Ten pure AMN patients without cerebral involvement were studied. Motor evoked potentials (MEPs), somatosensory evoked potentials (SEPs), auditory brainstem response (ABR), and pattern reversal full-field visual evoked potentials (VEPs) were recorded. For MEP recording, single-pulse or double-pulse magnetic brainstem stimulation (BST) was also performed.
Results: Abnormal MEP was observed in all ten patients, abnormal SEP in all ten, abnormal ABR in nine, and abnormal VEP in only one. Brainstem latency was measured in three of the seven patients with central motor conduction time (CMCT) prolongation. The cortical–brainstem conduction time was severely prolonged along the normal or mildly delayed brainstem–cervical conduction time in those three patients.
Conclusions: The pattern of normal VEP and abnormal MEP, SEP, ABR is a clinically useful electrophysiological feature for the diagnosis. BST techniques are helpful to detect, functionally, intracranial corticospinal tract involvement, probably demyelination, in pure AMN patients.

1. Introduction

X-linked adrenoleukodystrophy (ALD) is a peroxisomal disorder caused by mutation of the ABCD1 gene [1] whose biochemical abnormality is characterized by the accumulation of very long chain saturated fatty acids (VLCFA) [2–5]. The highly varied phenotype of X-linked ALD is classified into subtypes such as childhood cerebral ALD, adolescent cerebral ALD, adrenomyeloneuropathy (AMN), adult cerebral ALD, olivo-ponto-cerebellar ALD and Addison's disease-only ALD [6–8]. No correlation exists between phenotypes and genotypes [9]. The central nervous system pathology comprises two apparently disparate types of cerebral form (cerebral ALD) and AMN. The cerebral ALD is characterized by a severe inflammatory demyelinating lesion in the cerebrum (myelinopathy) [10]. The AMN is characterized by distal axonopathy: degeneration of spinal tracts distributed in a 'dying-back' pattern [11]. These two major forms of the disease differ fundamentally with respect to their prognoses. Although rapidly progressive cerebral ALD engenders total disability during the first decade, some patients with AMN survive to the eighth decade [6]. However, about half of the AMN patients clinically develop cerebral involvement within 10 years after onset [7,8]. The patients without cerebral involvement are referred to as "pure" AMN, whereas the patients with cerebral involvement are referred to as "cerebral" AMN. The magnetic resonance image (MRI) in pure AMN is often normal but may show changes up to the internal capsule [12,13], and the corticospinal tract lesions in pure AMN are considered to be axonal pathology [12,14,15]. On the other hand, the pathological mechanism in cerebral AMN is proposed to be the cerebral demyelination in addition to the distal axonopathy [12]. Therefore, the assessment of brain function using neurophysiological methods is very important in considering prognosis and possible treatment in AMN patients [6].

Central efferent function is physiologically examined using motor evoked potential (MEP). In fact, MEP studies have revealed frequent abnormal central motor conduction in AMN patients [16–18]. The central motor conduction time (CMCT) mainly reflects the overall function of the motor tract of the central nervous system. However, it does not indicate the level of motor tract involvement: whether it is intracranial, extracranial, or both. We previously developed methods to activate the descending motor tracts at the level of the pyramidal decussation (foramen magnum) using electrical stimulation [19] and magnetic stimulation [20]. The methods [brainstem stimulation (BST)] have been shown to be clinically useful for localizing corticospinal tract lesions in patients with various neurological disorders [21–25]. For this investigation, we applied this stimulation along with cortical and spinal stimulations to show

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Table 1
Background of patients.

Case	Age	ABCD1 mutation	Disease duration (years)	Main symptom(s)	Brain MRI	Loes score	Spinal MRI
1	32	Missense (H667N)	1	Spastic gait, pigmentation	Normal	0	Normal
2	44	Nonsense (W595X)	1	Spastic gait, sensory disturbance (leg)	Normal	0	Atrophy
3	61	N.E.	4	Spastic gait, muscular weakness (leg), sensory disturbance (leg)	Normal	0	Normal
4	30	Missense (S290W)	5	Spastic gait, sensory disturbance (leg), dysuria, dyschezia, impotence	P, V	2.5	Normal
5	31	Missense (F540S)	5	Spastic gait	V	0.5	Normal
6	24	Missense (A616D)	6	Spastic gait, sensory disturbance (leg), dysuria, impotence	Normal	0	Normal
7	31	Frameshift (Y281)	8	Spastic gait, sensory disturbance (leg), dyschezia	C	0.5	Normal
8	33	Missense (G277R)	8	Spastic gait, dysuria, dyschezia impotence	P	2	Atrophy
9	58	N.E.	18	Spastic gait, dysuria	Normal	0	Atrophy
10	58	N.E.	19	Spastic gait, impotence	Normal	0	Normal

MRI: magnetic resonance image, P: pyramidal system, V: visual pathway, C: cerebellum, N.E.: not examined.

which part of the descending tract was affected. We also adopted a recently reported powerful stimulation method to evoke clear MEPs in patients without any MEPs to single-pulse BST: double-pulse magnetic BST [26].

The central afferent functions are usually studied with various evoked potentials such as somatosensory evoked potential (SEP), auditory brainstem response (ABR), and visual evoked potential (VEP). These three evoked potentials also have shown afferent system conduction abnormalities in AMN patients [16,17,27–32].

The aim of this study is to investigate efferent and afferent conductions of the central nervous system in pure AMN patients using the four types of evoked potentials including magnetic BST. Some results of MEPs in this study were described in a previous report [26].

2. Methods

2.1. Patients

We studied ten male patients with AMN. Based on the course of the disease and clinical symptoms, they were diagnosed as AMN. Plasma VLCFA was abnormally increased in all of them. The ABCD1 gene mutation was analyzed in seven patients (cases 1, 2, 4-8) after receiving their informed consent [33,34]. Their patient characteristics and clinical features are presented in Table 1. Their ages were 24–61 years (mean ± SD, 40.2 ± 13.9 years). Their body heights were 165–175 cm (169.1 ± 3.2 cm). The durations of illness at the time of our experiment were 1–19 years (7.5 ± 6.3 years). All patients presented with spastic paraplegia with positive Babinski signs. Five patients presented with diminished superficial and deep sensation in the lower extremities (cases 2–4, 6, and 7). On the other hand, all patients presented no symptoms of motor and sensory systems in the upper extremities. They all also had no auditory and visual complaints. Brain and spinal MRIs were also taken. The lesions observed on brain MRI were described according to a previous paper [35]. Because all patients had no clinical or radiological cerebral involvement, all of them were classified into pure AMN [12,13]. Both MEP and SEP were recorded on the more affected side of motor symptom; ABR and VEP were recorded on both sides. We compared the latencies of these evoked potentials with the normal values in our institution.

Informed consent to participate in this study was obtained from all patients. The protocol was approved by the Ethics Committee of the University of Tokyo. It was conducted in accordance with the ethical standards of the Declaration of Helsinki.

2.2. MEP recording

Patients were seated comfortably on a reclining chair. MEPs were recorded from the first dorsal interosseous (FDI) and tibialis anterior (TA) muscles with pairs of Ag/AgCl surface cup electrodes placed in a belly tendon montage. Signals were fed to an amplifier (Biotop; GE Marquette Medical System, Japan) with filters set at 100 Hz and 3 kHz; the signals were recorded using software (TMS bistim tester; Medical Try System, Japan) for later off-line analyses.

Magnetic stimulation was conducted using a monophasic stimulator (Magstim 200; The Magstim Co. Ltd., UK) for transcranial magnetic stimulation (TMS), magnetic spinal motor root stimulation, and single-pulse BST. Double-pulse BST was given by connecting the two magnetic stimulators linked with a Bistim module (The Magstim Co. Ltd., UK).

For both muscles, CMCT was measured in each patient. For FDI, the onset latency of MEP elicited by TMS over the contralateral hand motor area using a round coil (10 cm diameter; The Magstim Co. Ltd., UK) was measured in the active condition (cortical latency). Induced current flowed in the posterior to the anterior direction over the hand motor area [36,37]. For TA, cortical latency was measured placing a double-cone-coil (The Magstim Co. Ltd., UK) [38] over the Cz (international 10–20 system), with induced current flowing mediolaterally over the leg motor area [39]. The onset latency of MEP to magnetic spinal motor root stimulation was also measured by activating cervical and lumbar spinal nerves with a round coil (10 cm diameter) placed over the spinal enlargement (spinal latency) [40,41]. The CMCT was calculated by subtracting the spinal latency from the cortical latency [37].

For FDI, single-pulse BST was also performed in active and relaxed conditions [20]. For BST, a double-cone-coil was placed with the center of the junction region over the inion. The coil current flowed downward at the junction of the coil so that the maximal current induced in the head flowed upward because this current direction has the lowest threshold for evoking MEPs [23]. The onset latency of MEP to single-pulse BST was measured (brainstem latency). When a single-pulse BST with maximal stimulator output was insufficient to evoke any MEP, double-pulse BST at an interstimulus interval of 2 ms was tried in a relaxed condition [26]. The stimulus intensities of double-pulse BST were set at the maximal stimulator output. The onset latency of MEP to double-pulse BST was measured from the time of the second pulse, which was identical to that of single-pulse BST (brainstem latency) [26]. The cortical–brainstem and brainstem–cervical conduction times were obtained, respectively, by subtracting the brainstem latency from the cortical latency and the spinal latency from the brainstem latency.

2.3. SEP recording

For this study, the SEPs were elicited after electrical stimulation (a constant current square wave pulse with duration of 0.2 ms) of the median nerve at the wrist or posterior tibial nerve at the ankle, as described in previous reports [16]. For recording N13 and

Table 2
Results of one efferent evoked potential.

Case	Side	MEP (FDI) cervical	CMCT	Cortical–BST	BST–cervical	MEP (TA) lumbar	CMCT (ms)
1	Rt	13.9	7.2	2.9	4.3	14.3	**21.0**↑
2	Rt	12.5	**8.6**↑	N.E.		12.1	**23.3**↑
3	Lt	**17.3**↑	6.0	1.7	4.3	**17.1**↑	**19.5**↑
4	Rt	12.4	**13.0**↑	**7.8**↑	**5.2**↑	12.8	**30.1**↑
5	Lt	13.5	**15.4**↑	**N.D.**		15.1	**N.D.**
6	Rt	14.0	**12.8**↑	**N.D.**		11.8	**N.D.**
7	Lt	12.0	**11.3**↑	**N.D.**		11.6	**N.D.**
8	Rt	14.3	**8.6**↑	**5.1**↑	3.5	14.8	**26.8**↑
9	Rt	14.7	**10.3**↑	**6.2**↑	4.1	**17.0**↑	**18.7**↑
10	Rt	12.8	7.0	3.2	3.8	15.6	**21.3**↑
Normal values (upper limit, +2.5 SD)		15.1	8.0	4.1	5.0	16.7	17.8

MEP: motor evoked potential, FDI: first dorsal interosseous, TA: tibialis anterior, CMCT: central motor conduction time, cortical–BST: cortical–brainstem conduction time, BST–cervical: brainstem–cervical conduction time, ↑: prolonged latency, N.E.: not examined, N.D.: not detected, bold type: abnormal findings.

N20 potentials elicited by median nerve stimulation, the electrodes were placed at two locations: the spinous process of C6 and C3′ or C4′ (2 cm posterior to the C3 or C4, the international 10–20 system), with Fz reference. For recording N21 and P38 potentials evoked by tibial nerve stimulation, the recording electrodes were placed at two locations: the spinous process of L1 with contralateral iliac crest reference, and Cz′ (2 cm posterior to the Cz) with Fz reference. For the median nerve SEP, the peak latency of N13 and the inter-peak latency of N13–N20 were measured, and for the tibial nerve SEP, the N21 peak latency and the N21–P38 inter-peak latency were measured. The inter-peak latencies, N13–N20 and N21–P38, are conventionally called the cortical sensory conduction time (CSCT) whereas the peak latencies, N13 and N21, are called the peripheral conduction time [16,42].

2.4. ABR recording

The ABR was recorded as reported in our previous report [43]. The recording electrode was placed over the vertex (Cz) and the reference electrode on the unilateral earlobe, and the ground electrode was over the Fz. An 80 dB (equivalent sound pressure level, 100 μs duration, alternating) click sound was given to the unilateral ear on the reference side at a rate of 5 Hz with a headphone; both sides were examined separately. The peak latencies of I and V waves were measured, and the inter-peak latency between I and V waves was calculated. The neural generator of I wave in humans is considered as the acoustic nerve; that of the V wave is the auditory interneurons at the level of the inferior colliculus [44]. Therefore, the inter-peak latency of I–V waves is mainly expected to reflect the central auditory conduction.

2.5. VEP recording

Monocular pattern reversal full-field VEP was recorded. A black-and-white checkerboard pattern placed 127 cm in front of the subjects was reversed at 1 Hz. The total stimulus visual angle and each check subtended angle of 16 × 12 degrees and 60 min, respectively. One eye was covered with an eye patch; both eyes were examined alternately. The three recording electrodes were placed in the mid-occipital (MO), in midline 5 cm above the inion, the left-occipital (LO), in left 5 cm of MO, and the right-occipital (RO), in right 5 cm of MO. A mid-frontal (MF) electrode placed 12 cm above the nasion as the references. The latency of the major positive peak of the VEP (P 100) was determined.

Table 3
Results of three afferent evoked potentials.

Case	Side	SEP (median)		SEP (tibial)		Side	ABR		VEP, P 100 (ms)
		N13	N13–N20	N21	N21–P38		I	I–V	
1	Rt	14.3	**7.1**↑	23.4	18.2	Lt	1.43	**5.58**↑	109.0
						Rt	1.41	**5.29**↑	109.0
2	Rt	13.6	**8.9**↑	21.6	**23.6**↑	Lt	1.48	**5.72**↑	106.5
						Rt	1.43	**5.75**↑	99.3
3	Lt	**16.8**↑	6.7	**29.8**↑	19.0	Lt	1.50	**5.14**↑	110.0
						Rt	1.45	**4.67**↑	109.8
4	Rt	13.3	**8.0**↑	20.9	**38.9**↑	Lt	1.52	**5.48**↑	107.1
						Rt	1.70	**5.16**↑	103.2
5	Lt	**15.5**↑	**7.5**↑	24.1	**31.8**↑	Lt	1.52	**5.49**↑	105.3
						Rt	1.89	**4.63**↑	106.8
6	Rt	15.1	**9.3**↑	20.5	**N.D.**	Lt	1.54	**5.08**↑	**149.4**↑
						Rt	1.49	**4.99**↑	**146.6**↑
7	Lt	14.3	**7.0**↑	21.0	**29.8**↑	Lt	1.57	**5.06**↑	97.0
						Rt	1.49	**4.83**↑	96.0
8	Rt	14.8	**7.2**↑	**24.6**↑	**25.0**↑	Lt	1.86	**5.39**↑	97.5
						Rt	1.57	**5.41**↑	99.3
9	Rt	**16.2**↑	6.4	**26.3**↑	19.8	Lt	1.33	**5.22**↑	113.7
						Rt	1.38	**4.81**↑	113.1
10	Rt	N.E		20.3	33.7↑	Lt	1.77	**4.25**	102.3
						Rt	1.78	4.10	94.5
Normal values (upper limit, +2.5 SD)		15.3	6.8	24.4	20.5		1.92	4.57	114.1

SEP: sensory evoked potential, VEP: visual evoked potential, ABR: auditory brainstem response, ↑: prolonged latency, N.E.: not examined, N.D.: not detected, bold type: abnormal findings.

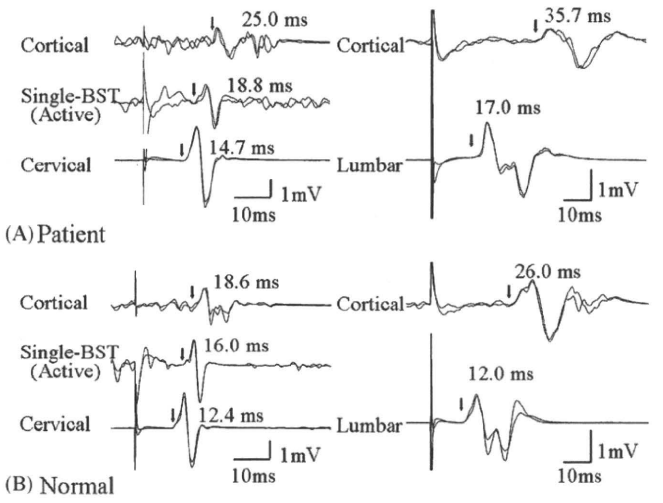

Fig. 1. MEP study in case 9. (A) MEP in a representative patient (case 9). Left figure shows MEPs recorded from FDI. CMCT is prolonged (10.3 ms, upper limit of normal values 8.0 ms). Only the cortical–brainstem conduction time is prolonged (6.2 ms, upper limit 4.1 ms), suggesting corticospinal tract involvement at the intracranial level. Right figure shows MEPs recorded form TA. CMCT is 18.7 ms (upper limit 17.8 ms) and spinal latency is 17.0 ms (upper limit 16.7 ms), indicating both central and peripheral motor conduction delays. (B) MEP in a normal subject.

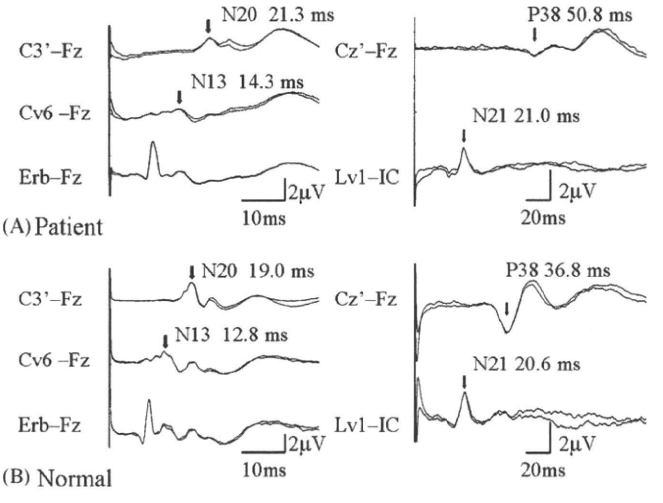

Fig. 2. SEP study in case 7. (A) SEP in a representative patient (case 7). Left figure shows the median nerve SEP. The bottom traces show the responses at the ipsilateral Erb's point to record peripheral nerve volley. N13–N20 latency is 7.0 ms (upper limit of normal values 6.8 ms) and N13 latency is 14.3 ms (upper limit 15.3 ms), indicating only CSCT prolongation. Right figure shows the tibial nerve SEP. CSCT (N21–P38 latency) is prolonged (29.8 ms, upper limit 20.5 ms). (B) SEP in a normal subject.

3. Results

The results of one efferent evoked potential and three afferent evoked potentials are presented in Tables 2 and 3. The P 100 latency of VEP was measured in the montage of MO–MF where the maximal amplitude was obtained in all ten patients.

Fig. 1 displays the waveforms of MEP in case 9 as an illustration. The CMCTs and spinal latencies for FDI and TA were measured in all AMN patients. For FDI, the CMCT was prolonged in seven patients (cases 2, 4, 5, 6, 7, 8, and 9). The spinal latency for FDI was prolonged in one patient (case 3). For TA, the CMCT was prolonged in seven patients or MEPs were not detected in the other three patients (cases 5, 6, and 7). The spinal latency for TA was prolonged in two patients (cases 3 and 9). Single-pulse or double-pulse BST was performed in nine of ten patients [one patient (case 2) declined to participate in the BST experiments]. Single-pulse BST elicited MEPs in five patients (cases 1, 3, 8, 9, and 10). Double-pulse BST was given to the other five patients, and evoked MEPs in one patient (case 4). Consequently, brainstem latency was measured in six patients (cases 1, 3, 4, 8, 9, and 10). In two patients (cases 8 and 9), the cortical–brainstem conduction time was prolonged (case 8: 5.1 ms, case 9: 6.2 ms, upper limit: 4.1 ms) but the brainstem–cervical conduction time was normal (case 8: 3.5 ms, case 9: 4.1 ms, upper limit: 5.0 ms). In one patient (case 4), both the cortical–brainstem and brainstem–cervical conduction times were prolonged (7.8 ms and 5.2 ms, respectively) but prolongation of the former conduction time was predominant. In three patients (cases 1, 3 and 10) with normal CMCT, both conduction times were normal.

Fig. 2 displays the representative waveforms of SEP (case 7). In the median nerve SEP, the CSCT was prolonged in seven of nine patients studied (cases 1, 2, 4, 5, 6, 7, and 8). Median nerve SEP was not examined in one patient (case 10). The peripheral conduction was prolonged in three of them (cases 3, 5, and 9). Regarding the tibial nerve SEP, the CSCT was prolonged or SEPs were not evoked in seven patients (cases 2, 4, 5, 6, 7, 8, and 10); the peripheral conduction time was prolonged in three patients (cases 3, 8, and 9). Fig. 3 displays the waveforms of ABR and VEP in case 6 as a representative of cases. The inter-peak latency of I–V waves of ABR was prolonged in nine patients and normal in one patient (case 10). The I wave latency was within normal limit in all patients. The P 100 latency was prolonged in only one of ten patients (case 6).

Brain MRI revealed abnormalities in four patients (cases 4, 5, 7, and 8) and no abnormalities in the other six patients. The pyramidal tract lesions (cases 4 and 8: Loes score 2), questionable optic radiation lesions (cases 4 and 5: Loes score 0.5) and unilateral cerebellum lesions (case 7: Loes score 0.5) were observed. Cerebral white matter was preserved in all patients. Spinal MRI showed atrophy of the spinal cord in three patients (cases 2, 8, and 9) and no abnormalities in the other patients.

MEP and SEP abnormalities were observed in all patients, although only four patients (cases 2, 4, 8, and 9) exhibited MRI abnormalities in the motor and sensory pathways (pyramidal tract lesions or spinal cord atrophy). ABR abnormalities were observed in nine patients except one patient (case 10), although no auditory

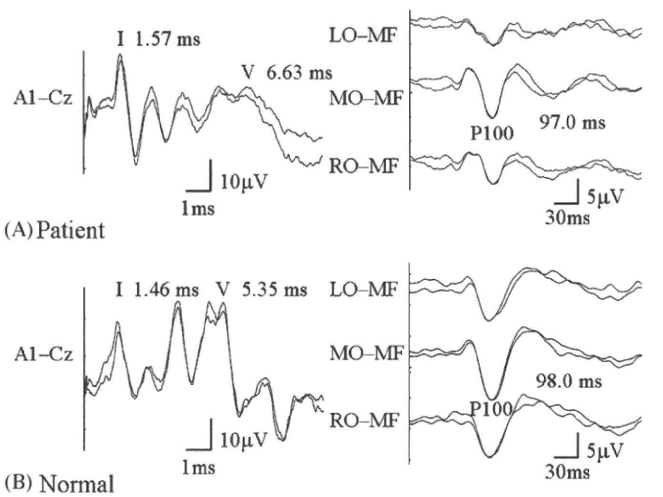

Fig. 3. ABR and VEP studies in case 7. (A) ABR and VEP in a representative patient (case 7). Left figure shows ABR waveforms in A1-Cz montage evoked by left sound stimulation. I wave latency is normal (1.57 ms, upper limit of normal values 1.92 ms), whereas I–V latency is prolonged (5.06 ms, upper limit 4.57 ms), suggesting the central auditory conduction delay. Right figure shows the VEP waveforms evoked by left monocular full-field stimulation. The maximal amplitude is obtained in the MO–MF montage. The P 100 latency is within normal limit (97.0 ms, upper limit 114.1 ms). (B) ABR and VEP in a normal subject.

pathway lesions were found in any of the ten patients. VEP abnormalities were seen in only one patient (case 6) whose brain MRI showed no visual pathway lesions.

Analyses of these patients yielded important physiological results. Every evoked potential revealed an abnormal conduction, even though MRIs showed normal findings. Regarding central efferent conduction, the cortical–brainstem conduction time was much more prolonged than the brainstem–cervical conduction time (cases 4, 8, and 9).

4. Discussion

This study revealed several physiological features in pure AMN patients. Although motor and sensory functions in the upper extremities were normal in all patients, both MEP and SEP in the upper extremities exhibited abnormal findings in eight patients. Similarly, despite normal auditory and visual functions, ABR and VEP could depict abnormalities, in nine patients and one patient, respectively. Thus, evoked potentials can detect subclincial lesions in the central nervous systems [45]. In addition, evoked potentials often revealed functional abnormalities of efferent and afferent conductions, even before any changes in MRI were evident, which is compatible with results described in the relevant literature [16,28,29,31].

The frequencies with which these evoked potentials detected abnormal conductions differed among these methods: MEP, SEP, and ABR were, respectively, abnormal in ten, ten, and nine of ten patients. In contrast, VEP was abnormal in only one out of ten patients. Therefore, MEP, SEP, and ABR abnormalities were more often observed than those of VEP. In fact, MEP, SEP, and ABR abnormalities are frequent findings in AMN patients [16,17,27,28,30–32]. In contrast, the incidence of pattern reversal full-field VEP abnormalities is not high in AMN patients (only 15 out of 59 AMN patients, 25.4%) [29]. Therefore, our studies verified the assumption from the prior studies, and we can regard normal pattern-reversal full-field VEP and abnormal MEP, SEP, and ABR patterns as an important neurophysiological feature that is frequently observed in this disorder.

In central efferent conduction, BST studies revealed severely delayed cortical–brainstem conduction time along the normal or mildly delayed brainstem–cervical conduction time in three pure AMN patients. Single-pulse BST failed to evoke MEPs in four out of nine patients. Single-pulse BST usually can evoke MEPs in FDI in healthy volunteers [20,26]. Therefore, the result implies that the threshold for BST was abnormally high in this disorder. Double-pulse BST was useful to detect these conduction delays, even in patients with no MEPs to single-pulse BST. Here we discuss the clinical significances of a newly discovered physiological feature in pure AMN: severely delayed cortical–brainstem conduction time.

Several mechanisms of severely delayed cortical–brainstem conduction time are considered to contribute to the prolongation of these conduction times [21,46]: (i) slowing of conduction in corticospinal tract fibers of large diameter (e.g. demyelinating disease), (ii) reduction in size (and number) of excitatory postsynaptic potentials generated by cortical or brainstem stimulation (e.g. amyotrophic lateral sclerosis), and (iii) reduction in the number of descending volleys induced by cortical stimulation caused by damage of cortical interneurons (e.g. cerebrovascular disease). Whatever the mechanism, the prolongation of the cortical–brainstem and brainstem–cervical conduction time can be taken to suggest that the corticospinal tract was affected, respectively, at the intracranial and extracranial levels [21].

Based on these discussions, we conclude that BST techniques are helpful to detect, functionally, intracranial corticospinal tract involvement in pure AMN patients. However, the prominent intracranial motor tract involvement in pure AMN patients cannot be explained solely by the main pathological mechanism, i.e. predominant spinal cord lesions (distal axonopathy). Consequently, we consider that the physiological results in the BST study probably indicate demyelination in the intracranial corticospinal tract for the following two reasons. About half of the pure AMN patients clinically develop the phenotype of cerebral AMN such as cerebral ALD [7,8]. In addition, autopsy studies of AMN patients have also shown mild intracranial demyelination [11]. Therefore, in other words, BST techniques might detect intracranial demyelination in pure AMN. For the proof of this possibility, more studies using various methodologies must be necessary.

5. Conclusion

The pattern of normal pattern reversal full-field VEP and abnormal MEP, SEP, and ABR is a clinically important neurophysiological feature for the diagnosis. The combination techniques of single-pulse and double-pulse BST are helpful to detect, functionally, intracranial corticospinal tract involvement, probably demyelination, in pure AMN patients.

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References

[1] Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, et al. Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. Nature 1993;361:726–30.
[2] Igarashi M, Schaumburg HH, Powers J, Kishimoto Y, Kolodny E, Suzuki K. Fatty acid abnormality in adrenoleukodystrophy. J Neurochem 1976;26(4):851–60.
[3] Tsuji S, Suzuki M, Ariga T, Sekine M, Kuriyama M, Miyatake T. Abnormality of long-chain fatty acids in erythrocyte membrane sphingomyelin from patients with adrenoleukodystrophy. J Neurochem 1981;36:1046–9.
[4] Moser HW, Moser AB, Frayer KK, Chen W, Schulman JD, O'Neill BP, et al. Adrenoleukodystrophy: increased plasma content of saturated very long chain fatty acids. Neurology 1981;31:1241–9.
[5] Tsuji S, Sano-Kawamura T, Ariga T, Miyatake T. Metabolism of [17,18-3H2] hexacosanoic acid and [15,16-3H2] lignoceric acid in cultured skin fibroblasts from patients with adrenoleukodystrophy (ALD) and adrenomyeloneuropathy (AMN). J Neurol Sci 1985;71:359–67.
[6] Moser HW. Adrenoleukodystrophy: phenotype, genetics, pathogenesis and therapy. Brain 1997;120:1485–508.
[7] Takemoto Y, Suzuki Y, Tamakoshi A, Onodera O, Tsuji S, Hashimoto T, et al. Epidemiology of X-linked adrenoleukodystrophy in Japan. J Hum Genet 2002;47:590–3.
[8] Suzuki Y, Takemoto Y, Shimozawa N, Imanaka T, Kato S, Furuya H, et al. Natural history of X-linked adrenoleukodystrophy in Japan. Brain Dev 2005;27:353–7.
[9] Takano H, Koike R, Onodera O, Sasaki R, Tsuji S. Mutational analysis and genotype–phenotype correlation of 29 unrelated Japanese patients with X-linked adrenoleukodystrophy. Arch Neurol 1999;56:295–300.
[10] Powers JM, Liu Y, Moser AB, Moser HW. The inflammatory myelinopathy of adreno-leukodystrophy: cells, effector molecules, and pathogenetic implications. J Neuropathol Exp Neurol 1992;51:630–43.
[11] Schaumburg HH, Powers JM, Raine CS, Spencer PS, Griffin JW, Prineas JW, et al. Adrenomyeloneuropathy: a probable variant of adrenoleukodystrophy. II. General pathologic, neuropathologic, and biochemical aspects. Neurology 1977;27:1114–9.
[12] Dubey P, Fatemi A, Huang H, Nagae-Poetscher L, Wakana S, Barker PB, et al. Diffusion tensor-based imaging reveals occult abnormalities in adrenomyeloneuropathy. Ann Neurol 2005;58:758–66.
[13] Dubey P, Fatemi A, Barker PB, Degaonkar M, Troeger M, Zackowski K, et al. Spectroscopic evidence of cerebral axonopathy in patients with "pure" adrenomyeloneuropathy. Neurology 2005;64:304–10.

[14] Loes DJ, Fatemi A, Melhem ER, Gupte N, Bezman L, Moser HW, et al. Analysis of MRI patterns aids prediction of progression in X-linked adrenoleukodystrophy. Neurology 2003;61:369–74.
[15] Kumar AJ, Köhler W, Kruse B, Naidu S, Bergin A, Edwin D, et al. MR findings in adult-onset adrenoleukodystrophy. Am J Neuroradiol 1995;16:1227–37.
[16] Ugawa Y, Kohara N, Shimpo T, Mannen T. Central motor and sensory conduction in adrenoleukomyeloneuropathy, cerebrotendinous xanthomatosis, HTLV-1-associated myelopathy and tabes dorsalis. J Neurol Neurosurg Psychiatry 1988;51:1069–74.
[17] Restuccia D, Di Lazzaro V, Valeriani M, Oliviero A, Le Pera D, Barba C, et al. Abnormalities of somatosensory and motor evoked potentials in adrenomyeloneuropathy: comparison with magnetic resonance imaging and clinical findings. Muscle Nerve 1997;20:1249–57.
[18] Restuccia D, Di Lazzaro V, Valeriani M, Oliviero A, Le Pera D, Barba C, et al. Neurophysiologic follow-up of long-term dietary treatment in adult-onset adrenoleukodystrophy. Neurology 1999;52:810–6.
[19] Ugawa Y, Rothwell JC, Day BL, Thompson PD, Marsden CD. Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. Ann Neurol 1991;29:418–27.
[20] Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I. Magnetic stimulation of corticospinal pathways at the foramen magnum level in humans. Ann Neurol 1994;36:618–24.
[21] Ugawa Y, Genba K, Mannen T, Kanazawa I. Stimulation of corticospinal pathways at the level of the pyramidal decussation in neurological disorders. Brain 1992;115:1947–61.
[22] Ugawa Y, Genba-Shimizu K, Kanazawa I. Electrical stimulation of the human descending motor tracts at several levels. Can J Neurol Sci 1995;22:36–42.
[23] Ugawa Y, Uesaka Y, Terao Y, Suzuki M, Sakai K, Hanajima R, et al. Clinical utility of magnetic corticospinal tract stimulation at the foramen magnum level. Electroencephalogr Clin Neurophysiol 1996;101:247–54.
[24] Ugawa Y. Stimulation at the foramen magnum level. Electroencephalogr Clin Neurophysiol 1999;51:S65–75.
[25] Ugawa Y. Stimulation at the foramen magnum level as a tool to separate cortical from spinal cord excitability changes. Adv Clin Neurophysiol (Suppl Clin Neurophysiol) 2002;54:216–22.
[26] Matsumoto H, Hanajima R, Hamada M, Terao Y, Yugeta A, Inomata-Terada S, et al. Double-pulse magnetic brainstem stimulation: mimicking successive descending volleys. J Neurophysiol 2008;100:3437–44.
[27] Tobimatsu S, Fukui R, Kato M, Kobayashi T, Kuroiwa Y. Multimodality evoked potentials in patients and carriers with adrenoleukodystrophy and adrenomyeloneuropathy. Electroencephalogr Clin Neurophysiol 1985;62:18–24.
[28] Aubourg P, Adamsbaum C, Lavallard-Rousseau MC, Lemaitre A, Boureau F, Mayer M, et al. Brain MRI and electrophysiologic abnormalities in preclinical and clinical adrenomyeloneuropathy. Neurology 1992;42:85–91.
[29] Kaplan PW, Kruse B, Tusa RJ, Shankroff J, Rignani J, Moser HW. Visual system abnormalities in adrenomyeloneuropathy. Ann Neurol 1995;37:550–2.
[30] Kaplan PW, Tusa RJ, Rignani J, Moser HW. Somatosensory evoked potentials in adrenomyeloneuropathy. Neurology 1997;48:1662–7.
[31] van Geel BM, Assies J, Haverkort EB, Koelman JH, Verbeeten Jr B, Wanders RJ, et al. Progression of abnormalities in adrenomyeloneuropathy and neurologically asymptomatic X-linked adrenoleukodystrophy despite treatment with "Lorenzo's oil". J Neurol Neurosurg Psychiatry 1999;67:290–9.
[32] Pillion JP, Kharkar S, Mahmood A, Moser H, Shimizu H. Auditory brainstem response findings and peripheral auditory sensitivity in adrenoleukodystrophy. J Neurol Sci 2006;247:130–7.
[33] Takano H, Koike R, Onodera O, Tsuji S. Mutational analysis of X-linked adrenoleukodystrophy gene. Cell Biochem Biophys 2000;32:177–85.
[34] Takahashi Y, Seki N, Ishiura H, Mitsui J, Matsukawa T, Kishino A, et al. Development of a high-throughput microarray-based resequencing system for neurological disorders and its application to molecular genetics of amyotrophic lateral sclerosis. Arch Neurol 2008;65:1326–32.
[35] Loes DJ, Hite S, Moser H, Stillman AE, Shapiro E, Lockman L, et al. Adrenoleukodystrophy: a scoring method for brain MR observations. Am J Neuroradiol 1994;15:1761–6.
[36] Barker AT, Jalinous R, Freeston IL. Non-invasive stimulation of human motor cortex. Lancet 1985;1:1106–7.
[37] Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Dimitrijević MR, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroenchephalogr Clin Neurophysiol 1994;91:79–92.
[38] Terao Y, Ugawa Y, Sakai K, Uesaka Y, Kohara N, Kanazawa I. Transcranial stimulation of the leg area of the motor cortex in humans. Acta Neurol Scand 1994;89:378–83.
[39] Terao Y, Ugawa Y, Hanajima R, Machii K, Furubayashi T, Mochizuki H, et al. Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. Brain Res 2000;859:137–46.
[40] Ugawa Y, Rothwell JC, Day BL, Thompson PD, Marsden CD. Magnetic stimulation over the spinal enlargements. J Neurol Neurosurg Psychiatry 1989;52:1025–32.
[41] Ugawa Y, Kohara N, Shimpo T, Mannen T. Magneto-electrical stimulation of central motor pathways compared with percutaneous electrical stimulation. Eur Neurol 1990;30:14–8.
[42] Hamada M, Hanajima R, Terao Y, Sato F, Okano T, Yuasa K, et al. Median nerve somatosensory evoked potentials and their high-frequency oscillations in amyotrophic lateral sclerosis. Clin Neurophysiol 2007;118:877–86.
[43] Arai N, Enomoto H, Okabe S, Yuasa K, Kamimura Y, Ugawa Y. Thirty minutes mobile phone use has no short-term adverse effects on central auditory pathways. Clin Neurophysiol 2003;114:1390–4.
[44] Hashimoto I, Ishiyama Y, Yoshimoto T, Nemoto S. Brain-stem auditory-evoked potentials recorded directly from human brain-stem and thalamus. Brain 1981;104:841–59.
[45] Kukowski B. Magnetic transcranial brain stimulation and multimodality evoked potentials in an adrenoleukodystrophy patient and members of his family. Electroencephalogr Clin Neurophysiol 1991;78:260–2.
[46] Thompson PD, Day BL, Rothwell JC, Dick JP, Cowan JM, Asselman P, et al. The interpretation of electromyographic responses to electrical stimulation of the motor cortex in diseases of the upper motor neurone. J Neurol Sci 1987;80:91–110.

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Quadri-pulse stimulation (QPS) induced LTP/LTD was not affected by Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene

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ABSTRACT

It has been reported that the brain derived neurotrophic factor (BDNF) has some functional roles in inducing plasticity in the adult human brain and the Val66Met BDNF polymorphism affects the plasticity induction. In contrast, some long lasting effects were not fully induced in subjects with non-Val–Val polymorphism. In this communication, we retrospectively investigated whether this polymorphism affects the plastic changes induced by a newly developed stimulation method (quadripulse stimulation (QPS)) in 12 subjects. Both long-term potentiation (LTP) and long-term depression (LTD) like effects were induced by QPS for 30 min in any types of BDNF Val66Met polymorphisms. This finding presents a striking contrast to the previous results, which showed reduced long-term effects elicited by some other induction methods in subjects with non-Val–Val polymorphism. Although we are not able to make a final conclusion about the effect of Val66Met BDNF polymorphism on QPS because of the small number of subjects studied, QPS may be less affected by the BDNF polymorphism than several other protocols for inducing LTP/LTD-like effects in humans. Several possibilities may explain this difference. One candidate possibility is that QPS may be long enough for inducing the late LTP/LTD like effect whereas the other stimulation methods may be long enough for early but not enough for late LTP/LTD like effect. It is conspicuous that the QPS for 30 min does elicit stable bidirectional long-term effects even in subjects with non-Val–Val polymorphism of BDNF.

The brain-derived neurotrophic factor (BDNF), which is originally considered to have some important functions in the development of nervous systems in the fetal period and childhood, has been shown to have some roles in plasticity induction in the adult brain [2]. Its precursor peptide, pro-BDNF, has also been shown to play some roles in plasticity induction. The BDNF induces long-term potentiation (LTP), and pro-BDNF induces long term depression (LTD). Therefore, the bidirectional plasticity depends on whether BDNF or pro-BDNF is dominantly present [10]. Another impact of BDNF is the report that the Val66Met BDNF polymorphism has some influence on the activity-dependent BDNF secretion and its processing and also on human hippocampal function [4].

Several stimulation methods have been reported to induce LTP/LTD like effects on the human motor cortex, and their application to the treatment of neurological disorders is promising. We have described a newly developed stimulation method to induce long-term effects using monophasic pulses of TMS, which is called QPS (quadri-pulse stimulation) [5]. Its metaplastic changes were also induced by the priming stimulation over the primary motor cortex (M1) [5] and supplementary motor area (SMA) [6]. These results suggest that QPS can induce LTP/LTD like effects on M1. In the meantime, one paper recently demonstrated that the theta bust stimulation (one of long term effects inducing methods) did not induce significant plastic changes in the human motor cortex in some BDNF polymorphism carriers even though it induced significant changes in subjects with other BDNF polymorphisms [3]. This finding has been confirmed by a following study [1]. It also showed

Abbreviations: BDNF, brain derived neurotrophin factor; TMS, transcranial magnetic stimulation; LTP, long term potentiation; LTD, long term depression; QPS, quadripulse stimulation.

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that different stimulation methods were variously affected by BDNF polymorphism, and stressed the need to consider BDNF polymorphism in the interpretation of the long-term effects [1]. To explore whether this polymorphism affects QPS in a similar manner, we retrospectively compared the QPS effects between two groups of BDNF polymorphism.

We studied 12 subjects, who participated in our previous two studies [5,6] and gave written informed consent to take part in genetic analyses. As a parameter of QPS effect, in the present study, we use the relative motor evoked potential (MEP) size at 30 min after QPS5 or QPS50 to the baseline response before QPS (size ratio 30 min) as a representative value of LTP or LTD because we used this parameter to draw a relation between the inter-stimulus interval of QPS and its main effect [5,6]. We also compared the whole time courses of the LTP/LTD effects between subjects with different BDNF polymorphism. The genomic DNA was extracted from peripheral leukocytes by standard procedures. Polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism (RFLP) assay were performed to determine the genotype of the DNA sequence variant, Val/Met polymorphism of the BDNF gene as reported previously [8]. The subjects were classified into two groups according to the BDNF polymorphism: Val–Val group and non-Val–Val group (Val–Met or Met–Met). The size ratios at 30 min were compared between these two groups using Student's *t*-test. The time courses for LTP or LTD were independently compared between the two groups of subjects using two factorial analysis of variance (ANOVA) test using GROUP (Val–Val and non-Val–Val) as between-subject factor and TIME (5, 10, 15, 20, 25, and 30 min after the end of QPS) as within-subject factor. Post hoc analyses were performed using the Bonferroni method to compensate for multiple comparisons. Statistical analyses were performed using a commercial software SPSS (ver. 16.0). These procedures were approved by the Ethics Committees of Fukushima Medical University, the University of Tokyo and Chiba University.

The 12 participants were genotyped as follows: 5 participants were found to be homozygous for the Val allele (Val66Val), 5 were Val66Met heterozygotes, and 2 were homozygous for the Met allele. The two groups, therefore, consisted of 5 subjects with Val–Val polymorphism and 7 subjects with non-Val–Val polymorphism. In QPS5 experiments, the mean (±SD) size ratio at 30 min after QPS was 2.07 (±0.96) for the Val–Val group and 2.22 (±0.61) for the non-Val–Val group (Fig. 1A). In QPS50 experiments, they were 0.53 ± 0.45 for Val–Val and 0.70 ± 0.38 for non-Val–Val groups (Fig. 1A). These were not significantly different between the two groups (*t*-test: $P > 0.3$ for both QPS5 and QPS50). The time courses of LTP/LTD like effects are shown in Fig. 1B and C. ANOVA revealed no significant effect of GROUP on the size ratio ($P = 0.955$ for QPS5, $P = 0.735$ for QPS50). The TIME had a significant effect on the size ratio ($P = 0.046$ for QPS5, $P = 0.034$ for QPS50). No significant interaction between these factors was seen ($P = 0.535$ for QPS5, $P = 0.680$ for QPS50). Post hoc analyses corrected for multiple comparisons revealed that significant potentiation was elicited by QPS 5 (Bonferroni method: $P = 0.034, 0.037, 0.008, 0.017, 0.0005, 0.0008$ for 5, 10, 15, 20, 25, 30 min after QPS, respectively), and significant suppression by QPS 50 (Bonferroni method: $P = 0.004$, 0,006, 0.001, 0.0006, 0.003, 0.01 for 5, 10, 15, 20, 25, 30 min after QPS, respectively).

We showed that normal amount of LTP and LTD like effects were induced by QPS for 30 min in any types of BDNF Val66Met polymorphisms. This finding presents striking contrast to the previous two papers [1,3]. They showed that, in non-Val–Val polymorphism subjects, some long-term effects were reduced in TBS, transcranial direct current stimulation (tDCS), transcranial random noise stimulation (tRNS) and paired associative stimulation (PAS) protocols.

Several possibilities may explain this discrepancy between their and our results.

The difference in duration of stimulation may be one important factor to explain this discrepancy. The QPS protocols gave TMS pulses for 30 min, and during this period the subjects should keep the target muscle relaxed. On the other hand, iTBS takes only 190 s, and tDCS, tRNS or PAS takes 10–13 min in total. The importance of QPS duration has been shown in our previous paper [5]. In contrast to the bidirectional after-effects of QPS for 30 min, QPS5ms for 10, 20 and 40 min unaffected MEP sizes after the protocols even though some of them had a priming effect. Why is the duration of stimulation so important? Two observations may explain the importance of duration of stimulation. One is the time needed for protein synthesis contributing to the synaptic plasticity, and the other is the fact that some target muscle voluntary contraction abolishes LTP/LTD like effects in humans. Some protein synthesis needed for late LTP/LTD begins around 30 min after stimulation [11], and actual synaptic morphological changes were seen 30 min, at shortest, after the burst stimulation in slice experiments [13]. In QPS experiments, MEP size changes were first accessed 30 min after QPS stimulation onset. In the other methods, however, they were measured 190 s to 13 min after the onset of their protocols. Measurements at these short intervals after stimulation may miss the effects. In addition, it is well known that target muscle contraction during a protocol abolishes the long term effects induced by TBS [7]. In the following MEP measurements, unintentional target muscle contractions sometimes occur. These unintentional contractions should disturb some cascade for the long-term effects. In contrast, the subjects should keep the target muscle relaxed in QPS stimulation for 30 min. It ensures complete relaxation of the target muscle for 30 min. Combination of these two factors should explain the importance of stimulation duration in LTP/LTD like effects induction in humans. The BDNF polymorphism may have a comparatively strong influence on the secretion of BDNF but no influence on its function [4]. If the duration of repetitive stimulation or the duration of stimulation and complete relaxation after stimulation is long enough for inducing the secretion of the total amount of BDNF for later LTP or LTD cascade even though the secretion is slow, normal magnitude of LTP or LTD must eventually be induced even in subjects with non-Val–Val polymorphism of BDNF. The usual LTP/LTD induction protocols elicit BDNF secretion which enhances its secretion presynaptically and postsynaptically by themselves [9], and the BDNF secretion may continue even after the protocols have ceased. This cascade must be blocked at some part when voluntary contraction contaminates. Based on these arguments, we suppose that QPS for 30 min in a completely relaxed state may be long enough for stable LTP/LTD like effects to be induced even in subjects with non-Val–Val BDNF polymorphism.

The second possible explanation for the discrepancy is the strength of LTP/LTD like effects. The LTP/LTD like effects induced by QPS may be stronger than those by the other methods. If so, strong QPS effects may not be affected by the Val66Met polymorphism in BDNF. The weak priming effects by shorter duration QPS may be affected by this polymorphism.

Another possible explanation is that there are differences in some parts of the cascade for LTP/LTD like effects induction between several induction methods, and some of them are affected by BDNF polymorphism, and some other part may be affected by another polymorphism. Many investigations have studied the influence of BDNF Val66Met polymorphism on LTP/LTD like effects. It is well known that several other factors affect these long-term effects, such as dopamine or serotonine [12]. Some polymorphisms of dopaminergic, serotonergic or other genes may also affect QPS.

This study has a few limitations. The first is the small number of subjects studied. Our subjects were not sufficient to make a firm conclusion about the BDNF polymorphism effects on QPS

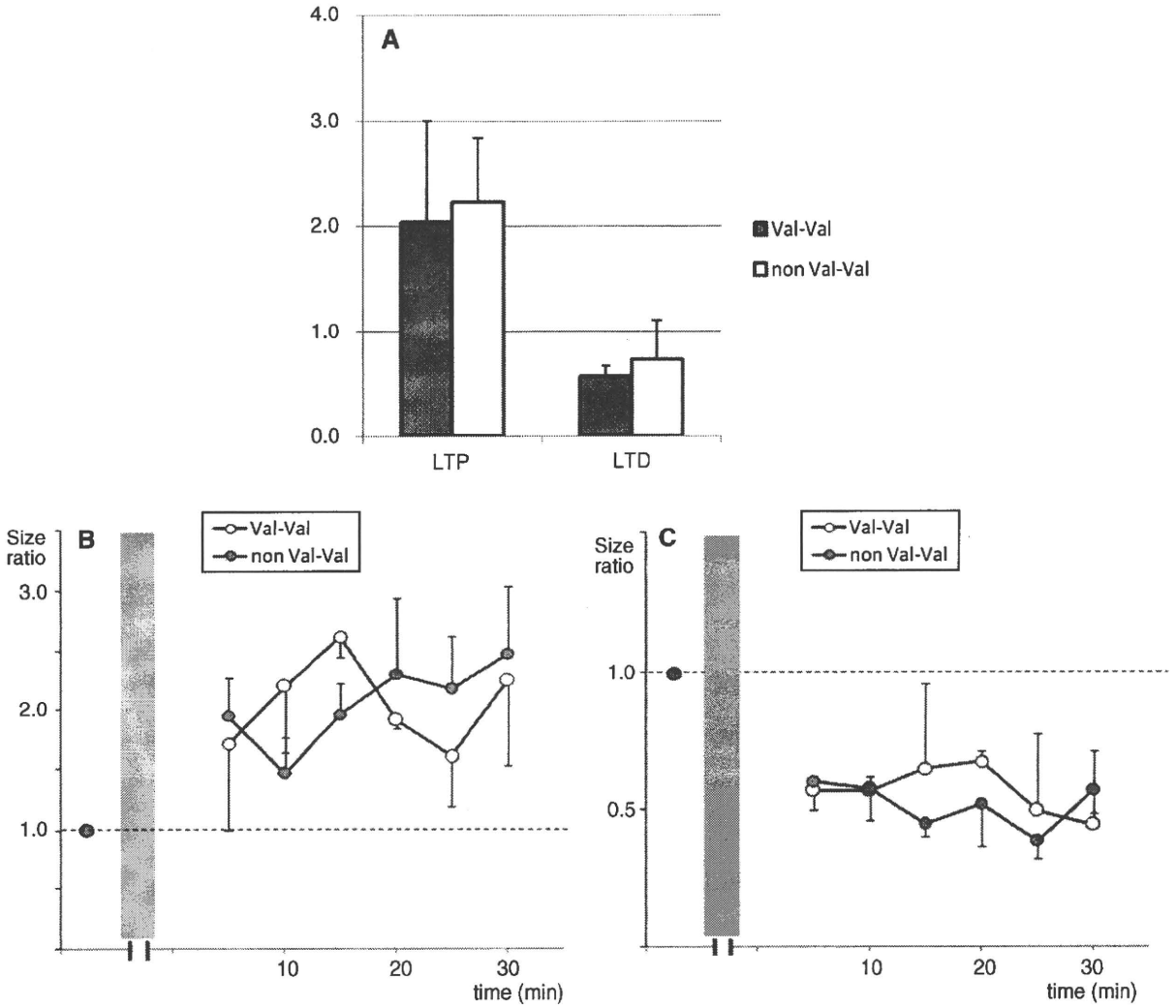

Fig. 1. (A) MEP size ratio at 30 min after QPS. The filled bars show the mean ± standard error (SE) size ratios after QPS5, and white bars those after QPS50. There were no significant differences between the subjects groups of Val–Val and non-Val–Val. (B) The mean ± standard error (SE) time courses of LTP like effects induced by QPS5 in Val–Val and non-Val–Val groups. Similar potentiation was induced in both groups of subjects. The time course did not significantly differ between the two groups of subject. (C) The mean ± standard error (SE) time courses of LTD like effects induced by QPS50 in Val–Val and non-Val–Val groups. Similar depression was induced in both groups of subjects. The time course did not significantly differ between the two groups of subject.

induced plasticity. We will study more subjects prospectively in the future. Another drawback is the lack of studies of effects of stimulation duration on QPS results. We should study several stimulation durations of QPS and compare their results. This is also one of the future research projects on QPS. As mentioned above, investigations of other genetic polymorphisms are also one of future projects.

Even with these limitations, it is a conspicuous point of this study that QPS for 30 min does elicit stable bidirectional long-term effects even in subjects with non-Val–Val polymorphism of BDNF.

Disclosures statement

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References

[1] A. Antal, L. Chaieb, V. Moliadze, K. Monte-Silva, C. Poreisz, N. Thirugnanasambandam, M.A. Nitsche, M. Shoukier, H. Ludwig, W. Paulus W. Brain-derived neurotrophic factor (BDNF) gene polymorphisms shape cortical plasticity in humans. Brain Stimulation, 2010 in press.
[2] C.R. Bramham, E. Messaoudi, BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis, Prog. Neurobiol. 76 (2005) 99–125.
[3] B. Cheeran, P. Talelli, F. Mori, G. Koch, A. Suppa, M. Edwards, H. Houlden, K. Bhatia, R. Greenwood, J.C. Rothwell, A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS, J. Physiol. 586 (2008) 5717–5725.
[4] M.F. Egan, M. Kojima, J.H. Callicott, T.E. Goldberg, B.S. Kolachana, A. Bertolino, E. Zaitsev, B. Gold, D. Goldman, M. Dean, B. Lu, D.R. Weinberger, The BDNF Val66Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function, Cell 11 (2003) 257–269.
[5] M. Hamada, Y. Terao, R. Hanajima, Y. Shirota, S. Nakatani-Enomoto, T. Furubayashi, H. Matsumoto, Y. Ugawa, Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation, J. Physiol. 586 (2008) 3927–3947.
[6] M. Hamada, R. Hanajima, Y. Terao, S. Okabe, S. Nakatani-Enomoto, T. Furubayashi, H. Matsumoto, Y. Shirota, S. Ohminami, Y. Ugawa, Primary motor cortical metaplasticity induced by priming over the supplementary motor area, J. Physiol. 587 (2009) 4845–4862.
[7] Y.Z. Huang, J.D. Rothwell, M.J. Edwards, R.S. Chen, Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human, Cereb. Cortex 18 (2008) 563–570.
[8] K. Itoh, K. Hashimoto, E. Shimizu, Y. Sekine, N. Ozaki, T. Inada, M. Harano, N. Iwata, T. Komiyama, M. Yamada, I. Sora, K. Nakata, H. Ujike, M. Iyo, Associ-

ation study between brain-derived neurotrophic factor gene polymorphisms and methamphetamine abusers in Japan, Am. J. Med. Genet B: Neuropsychiatr. Genet. 132B (2005) 70–73.
[9] V. Lessmann, K. Gottmann, M. Malcangio, Neurotrophin secretion: current facts and future prospects, Prog. Neurobiol. 69 (2003) 341–374.
[10] B. Lu, P.T. Pang, W.H. Woo, The Yin and Yang of neurotrophin action, Nat. Rev. Neurosci. 6 (2005) 603–614.
[11] Y. Lu, K. Christian, B. Lu, BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiol. Learn. Mem. 89 (2008) 312–323.
[12] V.S. Mattay, T.E. Goldberg, F. Sambataro, D.R. Weinberger, Neurobiology of cognitive aging: insights from imaging genetics, Biol. Psychol. 79 (2008) 9–22.
[13] U.V. Nagerl, N. Eberhrn, S.B. Cambridge, T. Bonhoeffer, Bidirectional activity-dependent report morphological plasticity in hippocampal neurons, Neuron 44 (2004) 759–767.

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Transcranial direct current stimulation over the motor association cortex induces plastic changes in ipsilateral primary motor and somatosensory cortices

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ABSTRACT

Objective: This study was performed to elucidate whether transcranial direct current stimulation (tDCS) over the motor association cortex modifies the excitability of primary motor (M1) and somatosensory (S1) cortices via neuronal connectivity.
Methods: Anodal, cathodal, and sham tDCS (1 mA) over the left motor association cortex was applied to 10 subjects for 15 min using electrodes of two sizes (9 and 18 cm^2). Both motor evoked potentials (MEPs) and somatosensory evoked potentials (SEPs) were recorded before, immediately after, and 15 min after tDCS. Electrode positions were confirmed by overlaying them on MRI anatomical surface images of two individuals.
Results: After applying anodal tDCS using the large electrode, amplitudes of MEP components significantly decreased, whereas those of early SEP components (N20 and P25) increase. Opposite effects were observed on MEPs and SEPs after cathodal tDCS. However, a small electrode did not significantly influence either MEPs or SEPs, irrespective of polarity. The small electrode covered mainly the dorsal premotor cortex (PMd) while the large electrode involved the supplementary motor area (SMA) in addition to PMd.
Conclusions: These results suggest that anodal tDCS over PMd together with SMA enhanced the inhibitory input to M1 and excitatory input to S1, and that cathodal tDCS might lead to an opposite effect.
Significance: The finding that only the large electrode modulated M1 and S1 implies that activation of PMd together with SMA by tDCS can induce plastic changes in primary sensorimotor areas.

1. Introduction

Nitsche and Paulus (2000) first described the efficacy of transcranial direct current stimulation (tDCS) for functional modulation of the human motor cortex. Since then, tDCS has become an increasingly useful technique for noninvasive brain stimulation that can be used not only to examine cortical function in healthy subjects but also to facilitate the treatment of various neurological disorders (Schlaug et al., 2008).

During tDCS, electrodes are placed and secured to the scalp over the desired areas and currents are delivered to the underlying cortical tissue. The direction of current flow determines the effects on the underlying tissue. When tDCS is applied over the primary motor cortex (M1), anodal tDCS (using the anodal electrode over M1 and the cathodal electrode over the contralateral orbit) enhances cortical excitability, which increases the amplitude of motor evoked potentials (MEPs). On the other hand, cathodal tDCS (using the cathodal electrode over M1) shows the opposite effect (Nitsche and Paulus, 2000).

In addition to studies of the direct functional effects of tDCS over M1 (Nitsche and Paulus, 2000; Nitsche et al., 2003a, 2007; Hummel et al., in press; Stagg et al., 2009) and the primary sensory cortex (S1) (Matsunaga et al., 2004; Dieckhöfer et al., 2006), which were spatially restricted, remote effects were observed after motor cortex–prefrontal tDCS in a positron emission tomography (PET) study (Lang et al., 2005). A recent study also reported that anodal tDCS over the premotor cortex (PM) reduced short-interval intracortical inhibition (SICI) at interstimulus interval (ISI) of 2 and 3 ms, and enhanced intracortical facilitation (ICF) at ISI of 10 and 15 ms and the MEP amplitude of ISI of 7 ms, while motor

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thresholds, single test-pulse MEPs, and input–output curves of MEPs remained unchanged (Boros et al., 2008). However, cathodal tDCS had no remarkable effect.

In repetitive transcranial magnetic stimulation (rTMS) studies, low-frequency rTMS (1 Hz) at 90% active motor threshold (AMT) over PM caused a long-lasting reduction in M1 excitability measured by single-pulse TMS (Gerschlager et al., 2001; Rizzo et al., 2003). In contrast, rTMS of 1 Hz at 80% AMT over PM had no after-effects on single test-pulse MEP but significantly facilitated ICI (Münchau et al., 2002). Interestingly, high-frequency rTMS (5 Hz) at 90% AMT resulted in the increase of M1 excitability with decreased ICI and vice versa at 80% AMT (Rizzo et al., 2003). Thus, the intensity and frequency of rTMS are important to induce up- or down-regulation of the excitability in distinct neuronal circuits of M1. However, the mechanism of remote effect by tDCS has not been fully elucidated. It is possible that rTMS and tDCS could share the mechanism of the modification of neuronal connectivity. Therefore, we examined the possibility that tDCS over PM modulates the functions of S1 and M1 in a manner similar to rTMS over PM which changes S1 excitability (Hosono et al., 2008). PM is specialized for the control of externally triggered motor actions (Mushiake et al., 1991) and is activated more strongly by symbolic cues (Schluter et al., 1998) and codes for hand and eye position (Pesaran et al., 2006). Our previous functional MRI study demonstrated that a significant relationship exists between PM and the sensorimotor cortex during externally guided movements (Tanizaki et al., 2003). However, the neuronal connectivity of PM–M1–S1 has not yet been directly evaluated. The aim of this study was therefore, to determine whether tDCS over PM modifies the excitability of ipsilateral M1 and S1 via cortico-cortical connectivity.

2. Methods

2.1. Subjects

Ten healthy college students (7 males, 3 females; mean age, 20.6 years; range, 20–21 years) participated in this study. None of them had received medical treatment for any condition. Informed consent was obtained before beginning the experiment, which was conducted according to the Declaration of Helsinki. The experimental procedures were also approved by the Ethics Committee of the Niigata University of Health and Welfare.

2.2. Experimental procedures

During the experiment, subjects were seated on a comfortable reclining armchair with a mounted headrest. All experimental protocols were performed in the neutral forearm position. To avoid carryover effects, all subjects participated in three experimental sessions on separate days that were at least a week apart. All subjects received cathodal, anodal, or sham tDCS (1 mA) using a large (4×4.5 cm; 18 cm^2) electrode over the left PM for 15 min in a counterbalanced order. As an additional experiment, tDCS over PM using a small (3×3 cm; 9 cm^2) electrode was performed in 6 of the 10 subjects. Because we performed this experiment to determine whether MEPs and somatosensory evoked potentials (SEPs) would change in a manner similar to that observed using the large electrode, we eliminated the sham condition in the experiment performed using the small electrode, and the subjects received only anodal and cathodal tDCS. MEPs from the right first dorsal interosseus (FDI) muscle with TMS over the left M1 and SEPs following right median nerve stimulation were recorded from the left C3′ (2 cm posterior to C3 of the International 10–20 system) before, immediately after, and 15 min after tDCS.

2.3. tDCS over the motor association cortex

In several previous rTMS studies, the stimulation site over PM was 2 cm anterior and 1 cm medial to the motor hotspot. This area was estimated as the dorsal PM (PMd) in a PET study (Fink et al., 1997). If the "conventional electrode (7×5 cm)" (Nitsche et al., 2007) is centered 2 cm anterior and 1 cm medial to the motor hotspot, the covered area could overlap the motor hotspot and cross the vertex. Therefore, the electrode size was reduced to 18 or 9 cm^2 in this study. The large electrode (4×4.5 cm = 18 cm^2) was centered 2 cm anterior and 3 cm medial to the motor hotspot, while the small electrode (3×3 cm = 9 cm^2) was centered 2 cm anterior and 1 cm medial to avoid covering the motor hotspot with the edge of the electrode (Fig. 1).

Conductive thick (0.3 cm) rubber rectangular electrodes covered with a saline-soaked sponge were placed on the scalp over the left PM. The reference electrode (4×4.5 cm = 18 cm^2) was placed on the contralateral forehead above the orbit. The scalp around PM was cleaned with alcohol, and electrode paste (Gelaid, Nihon Koden, Japan) was applied to reduce electrode impedance. Stimuli were applied using a constant current electrical stimulator (Eldith, NeuroConn GmbH, Germany).

Current strength was set at 1 mA for the large electrode but reduced to 0.5 mA for the small electrode to keep the current density constant (0.055 mA/cm^2). During stimulation, the DC current was initially increased in a ramp-like manner at 10-s intervals until current strength of 1 mA or 0.5 mA was obtained (Gandiga et al., 2006). tDCS was maintained for a total of 15 min. In sham experiments, tDCS was turned off after 30 s. The parameters for sham stimulation were derived from a previous report (Gandiga et al., 2006) in which perceived sensations on the skin, such as tingling, usually faded out during the first 30 s of tDCS. Under both conditions, the DC current was slowly turned off over the course of 10 s, out of the subject's view. This procedure did not elicit any perceived sensations. The session order was counterbalanced and subjects were blinded to tDCS conditions.

To prevent the subject from feeling pain, Nitsche et al. (2003b) recommended that current density should not exceed 0.02857 mA/cm^2. The current density used in this experiment (0.055 mA/cm^2) was higher than the recommended value. However, the current density in our study is comparable to that (0.05–0.057 mA/cm^2) used in previous studies in which side effects or pain sensation was not observed (Jeffery et al., 2007; Tanaka et al., 2009; Roche et al., 2010).

Exact stimulating electrode positions over the cortex were evaluated in 2 of the 10 subjects. Standard T1-weighted images were

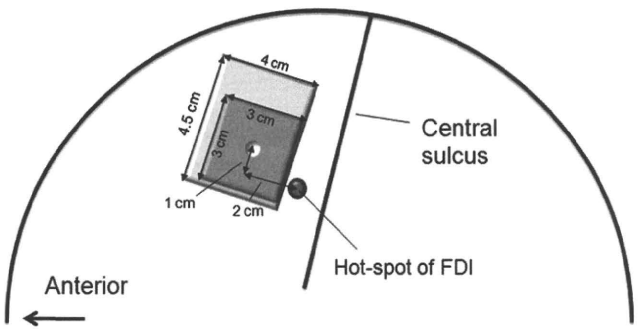

Fig. 1. Schematic illustration of the stimulus site over PM. The large electrode (4×4.5 cm = 18 cm^2) was centered 2 cm anterior and 3 cm medial to the motor hotspot, while the small electrode (3×3 cm = 9 cm^2) was centered 2 cm anterior and 1 cm medial to avoid covering the motor hotspot with the edge of the electrode. The reference electrode (4×4.5 cm = 18 cm^2) was placed on the contralateral forehead above the orbit.

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obtained (Signa HDi 1.5 T; GE Healthcare, Japan; 1.5 T, 1-mm slice), and electrode positions were confirmed by overlaying them on MRI anatomical surface images of each individual using a 3D magnetic space digitizer (Fastrak; Polhemus, USA) and specific software (Fusion; Shimazu Co. Ltd., Japan) (Maki et al., 1995; Okamoto et al., 2006; Shibusawa et al., 2009).

2.4. MEP recordings

MEPs elicited by TMS were recorded from the right FDI muscle. TMS was performed using a standard double (figure-of-eight) 70-mm coil connected to a monophasic Magstim 200 stimulator (Magstim, UK). The coil was placed tangentially to the scalp with the handle pointing posterolaterally 45 degrees from the midline. We determined the optimal position for activation of the right FDI muscle by moving the coil around the presumed hand motor area in M1 (approximately 4–6 cm lateral and 2 cm anterior to the vertex). The site at which TMS of slightly suprathreshold intensity consistently elicited the largest MEP in the FDI muscle was marked as the motor hotspot. The output of the stimulator was set to obtain an MEP of amplitude 1–1.5 mV (10% of M max) in the relaxed FDI muscle (Todd et al., 2006). TMS was delivered 12 times at 0.2 Hz with the subjects being asked to keep the muscle relaxed. Complete muscle relaxation was confirmed online via audiovisual feedback of electromyographic (EMG) activity.

Surface EMG was recorded using disposable silver–silver chloride surface electrodes. Recording and reference electrodes were placed over the muscle and the tendon. EMG signals were amplified (100×) and bandpass filtered (5–500 Hz) using a preamplification system (DL-140; 4 Assist, Japan) and digitized at 10 kHz (PowerLab; AD Instruments, Australia). Data were recorded and stored for off-line analysis (Scope; AD Instruments) on a personal computer.

2.5. SEP recordings

SEPs were recorded from the left parietal area during right median nerve stimulation at the wrist. A recording electrode was placed 2 cm posterior to C3 of the International 10–20 system. A reference electrode was placed on the right earlobe. SEPs were amplified with bandpass filters set at 0.2–1000 Hz and 300 responses were averaged. Brief electrical stimulation (0.2 ms) was delivered to the right median nerve at a frequency of 3 Hz (Neuropack Σ; Nihon Kohden, Japan). The stimulus intensity was fixed at about 1.2 times the motor threshold. SEP data were stored and analyzed using the same computer systems as those used for MEPs.

2.6. Data and statistical analysis

Peak-to-peak amplitudes of MEPs were measured after excluding trials with excessive artifacts. Mean MEP amplitudes were calculated from at least 8 of the 12 trials. Peak-to-peak amplitudes of four cortical SEP components, i.e., N20, P25, N33, and P45, were also analyzed. The amplitude of each component was measured from the preceding peaks.

Amplitudes of MEPs and SEPs were normalized to those recorded before tDCS. All data were expressed as means ± SEM and were statistically analyzed by two-way repeated measures analysis of variance (ANOVA) with the parameters polarity of tDCS (anodal vs. cathodal vs. sham) and time (before vs. immediately after vs. 15 min after). The sphericity of the data was tested by the Mauchly's test, and Greenhouse–Geisser corrected significance values were used when sphericity was lacking. Post hoc analysis was performed with Bonferroni's correction for multiple comparisons. A difference was accepted as significant at $p < 0.05$ for all analyses.

3. Results

3.1. MEPs after tDCS over the motor association cortex

Fig. 2 shows MEP waveforms recorded from a representative subject before, immediately after, and 15 min after anodal and cathodal tDCS over PM under the large electrode (18 cm^2) condition. The MEP amplitudes before tDCS in each stimulus condition were comparable: anodal 0.96 ± 0.06 mV, cathodal 0.94 ± 0.07 mV and sham 1.06 ± 0.07 mV, respectively. MEP amplitudes after anodal tDCS using the large electrode decreased markedly, whereas those after cathodal tDCS increased significantly. However, tDCS using the small electrode (9 cm^2) showed no remarkable effect.

Under the large electrode condition, two-way repeated measures ANOVA revealed a significant main effect of polarity of tDCS ($F_{1.92,\ 17.24} = 6.232$, $p = 0.01$) and interaction between polarity of tDCS and time ($F_{2.23,\ 20.69} = 8.211$, $p = 0.002$), but no significant main effect of time was observed. Post hoc analysis showed a significant difference between anodal and cathodal tDCS at both immediately after ($p < 0.001$) and 15 min after ($p = 0.027$) stimulation, and also between anodal and sham tDCS at immediately after stimulation ($p = 0.035$). A significant difference between before and 15 min after stimulation was observed for anodal tDCS ($p = 0.038$) as well as between before and immediately after stimulation for cathodal tDCS ($p < 0.001$). In contrast, no significant main effect or interaction was observed under the small electrode condition (Fig. 3).

3.2. SEPs after tDCS over the motor association cortex

Fig. 4 shows SEP waveforms recorded from a representative subject before, immediately after, and 15 min after anodal and

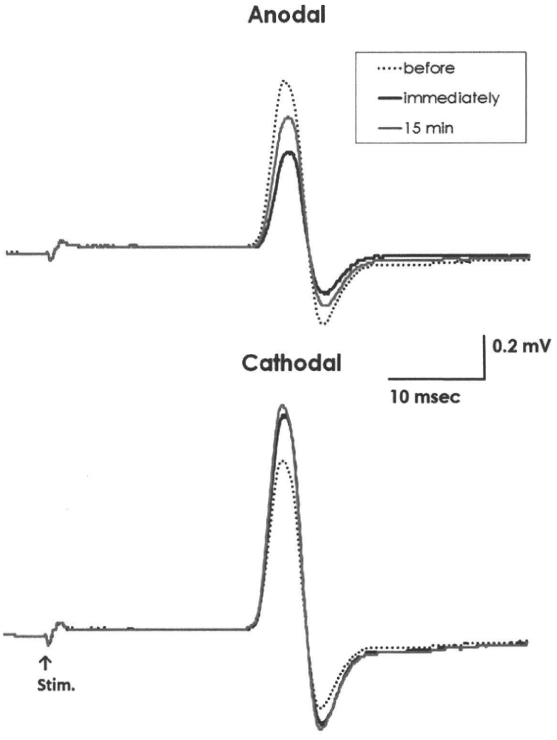

Fig. 2. MEP waveforms with TMS over M1 recorded from a representative subject before, immediately after, and 15 min after anodal and cathodal tDCS over the motor association cortex. The dotted lines represent MEPs prior to tDCS, black lines represent MEPs immediately after tDCS, and gray lines represent MEPs 15 min after tDCS. MEP amplitudes after anodal tDCS over the motor association cortex decreased, whereas those after cathodal tDCS increased.

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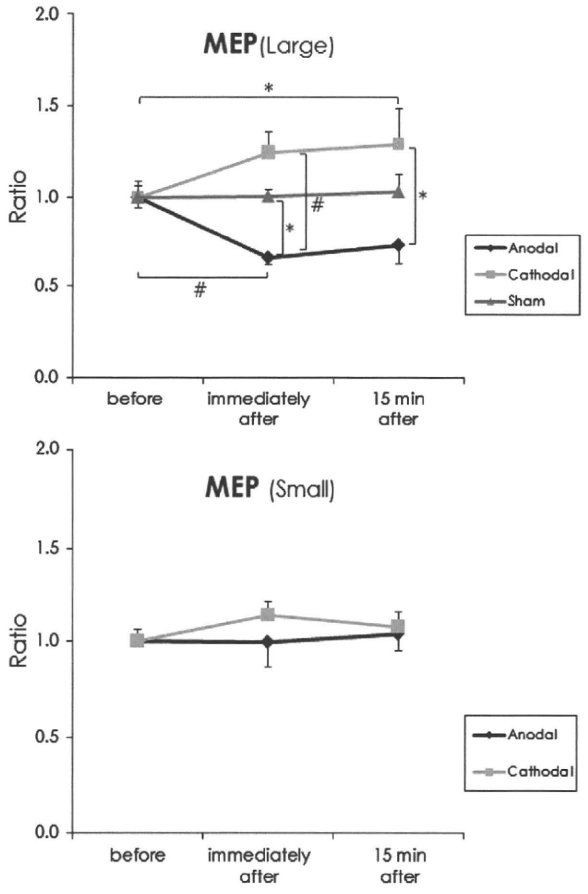

Fig. 3. Serial changes in MEP amplitudes before, immediately after, and 15 min after anodal, cathodal and sham tDCS under the large electrode condition (above), and anodal and cathodal tDCS under the small electrode condition (below). MEP amplitudes decreased markedly after anodal tDCS using the large electrode, whereas those after cathodal tDCS increased significantly. However, tDCS using the small electrode showed no remarkable effect. MEP amplitudes are normalized to those recorded before tDCS (mean ± SEM). ($^{*}p < 0.05$; $^{\#}p < 0.01$).

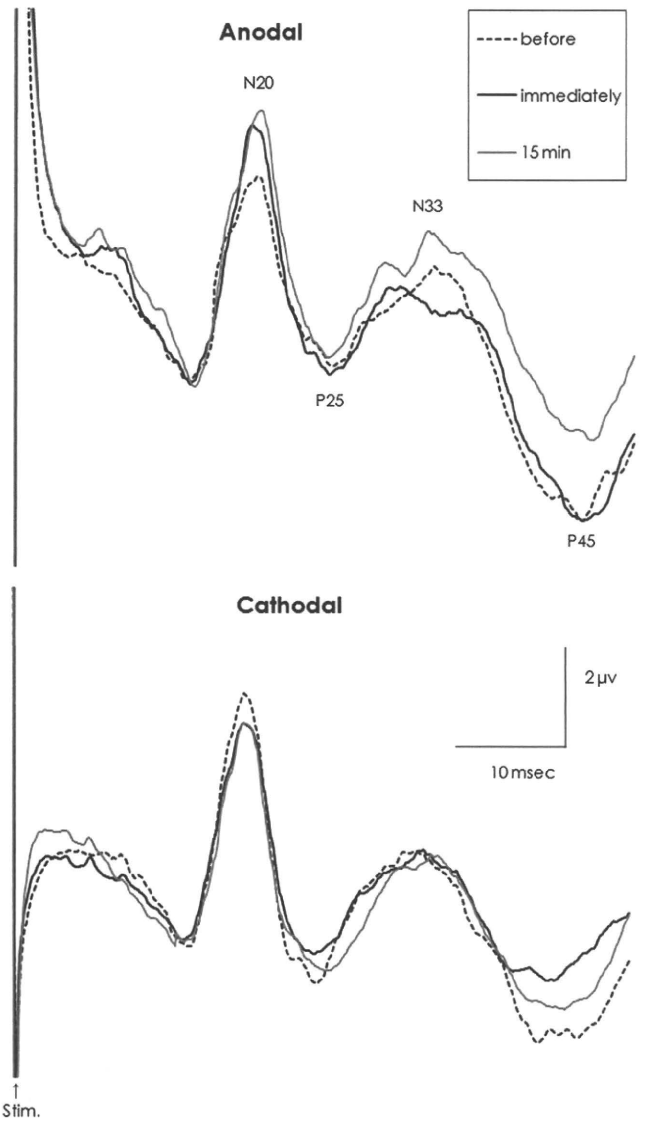

Fig. 4. SEP waveforms recorded from the left parietal area after right median nerve stimulation in a representative subject before, immediately after, and 15 min after anodal and cathodal tDCS over the motor association cortex. The dotted lines represent SEPs prior to tDCS, black lines represent SEPs immediately after tDCS, and gray lines represent SEPs 15 min after tDCS. SEP amplitudes increased after anodal tDCS but decreased after cathodal tDCS.

cathodal tDCS over PM under the large electrode condition. The amplitudes of N20 and P25 before tDCS in each stimulus condition were comparable: N20, anodal 3.37 ± 0.24 μV, cathodal 3.20 ± 0.23 μV and sham 3.62 ± 0.43 μV; P25, anodal 3.06 ± 0.34 μV, cathodal 3.22 ± 0.13 μV and sham 2.94 ± 0.09 μV, respectively. Contrary to the results obtained for MEPs, SEP amplitudes of N20 and P25 after anodal tDCS using the large electrode tended to increase, while those after cathodal tDCS decreased. However, tDCS using the small electrode showed no comparable effect.

Under the large electrode condition, two-way repeated measures ANOVA revealed a significant main effect of polarity ($F_{1.67,\ 15.02} = 11.702$, $p < 0.001$) and interaction between polarity and time ($F_{2.23,\ 20.11} = 3.819$, $p = 0.011$) for the N20 component. Post hoc analysis showed a significant difference between anodal and cathodal tDCS at both immediately after ($p = 0.013$) and 15 min after ($p = 0.01$) stimulation, between anodal and sham tDCS 15 min after stimulation ($p = 0.015$) and between cathodal and sham immediately after stimulation ($p = 0.024$). There was a significant main effect of polarity ($F_{1.12,\ 10.12} = 13.238$, $p < 0.001$) for the P25 component, but no main effect of time or interaction between polarity and time. Post hoc analysis revealed a significant difference between anodal and cathodal tDCS ($p < 0.001$), and between anodal and sham tDCS ($p = 0.013$) (Fig. 5). No significant effects of tDCS were observed for the N33 and P45 components. Under the small electrode condition, no significant main effect or interaction was observed for any component.

3.3. Stimulus sites

The position of the tDCS electrode was evaluated using a three-dimensional (3D) image (Fig. 6). Both large and small electrodes covered mainly PMd (Rizzolatti and Arbib, 1998; Bäumer et al., 2009) and a portion of the anterior part of PM (Civardi et al., 2001), but the large electrode further involved the supplementary motor area (SMA) without reaching ventral PM (PMv) or the contralateral hemisphere.

4. Discussion

This study demonstrated that tDCS over the motor association cortex using the large electrode modulated the excitability of ipsilateral M1 and S1. Interestingly, this manipulation induced opposite effects on M1 and S1. After anodal tDCS, amplitudes of MEPs significantly decreased compared with cathodal condition,

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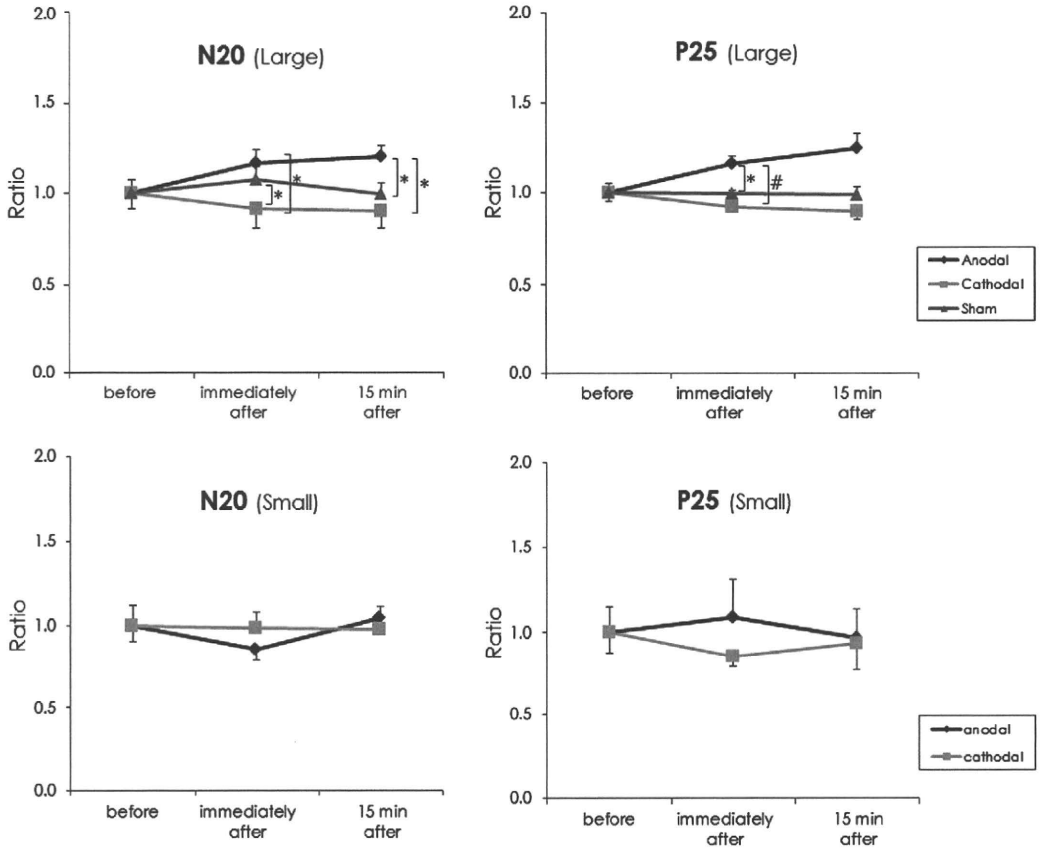

Fig. 5. Serial changes in SEP amplitudes (N20 and P25) before, immediately after, and 15 min after anodal, cathodal and sham tDCS under the large electrode condition (above), and anodal and cathodal tDCS under the small electrode condition (below). Contrary to the results for MEPs, N20 and P25 amplitudes after anodal tDCS using the large electrode increased compared with the cathodal condition, whereas those after cathodal tDCS decreased. However, tDCS using the small electrode showed no significant effect. SEP amplitudes are normalized to those recorded before tDCS (mean ± SEM). ($^{*}p < 0.05$; $^{\#}p < 0.01$).

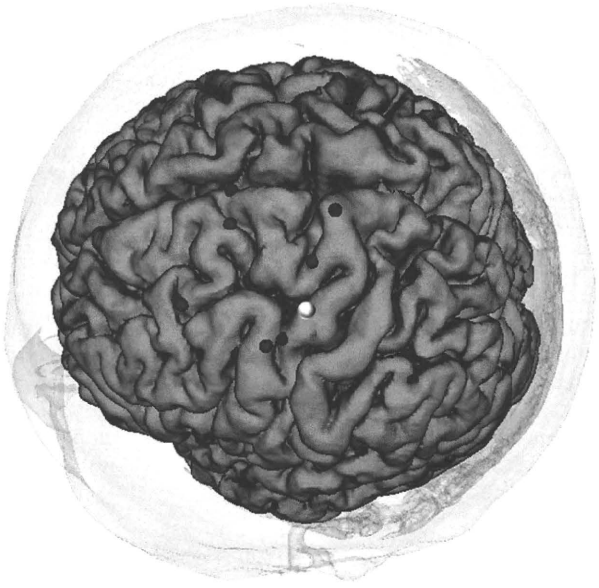

Fig. 6. tDCS electrode positions overlaid on MRI surface images. Edges of the electrodes are shown by red dots for the small electrode and blue dots for the large electrode. The white dot indicates the motor hotspot of FDI. Both the large and small electrodes covered mainly PMd and a portion of the anterior part of PM, while the large electrode also covered the supplementary motor area (SMA) but did not reach PMv and the contralateral hemisphere. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

whereas those of early SEP components (N20 and P25) increased. In contrast, the amplitudes of MEPs and SEPs did not show overt changes in the sham condition. Since the significant differences were observed between anodal/cathodal and sham conditions, the changes in M1 and S1 excitability can be caused by direct tDCS effect but not by changes in attention, habituation and fatigue resulted from repetitive measurements. Therefore, our result suggests that M1 was inhibited whereas S1 was excited by activation of PM and SMA after anodal tDCS. Opposite effects were observed after cathodal tDCS. However, the small electrode, which covered PMd but not SMA, did not significantly affect M1 or S1, irrespective of the polarity. Therefore, simultaneous modulation of PMd and SMA may alter the function of the sensorimotor network.

4.1. Changes in M1 excitability after tDCS over the motor association cortex

Nitsche and Paulus (2000) showed that anodal tDCS induced an excitatory effect, whereas cathodal tDCS produced an inhibitory effect over the cortex. In our study, presumed excitatory stimulation (anodal) over PMd and SMA reduced the MEP size, and inhibitory cathodal stimulation showed the opposite effect. Therefore, anodal tDCS activated the inhibitory connection to M1 whereas cathodal tDCS inhibited this connection, which in turn disinhibited M1.

Current density (current intensity/electrode size), duration, polarity, and location of stimulation have important implications in the neuromodulatory outcome (Zaghi et al., 2010). Nitsche et al. (2007) reported that the effect of tDCS did not diminish if

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the current density was held constant even when the size of the stimulation electrode and current strength were reduced. In this study, the current strength was set at 1 mA for the large electrode and was reduced to 0.5 mA for the small one to keep the current density constant (0.055 mA/cm^2). The effects on M1 excitability differed between the two electrodes. Thus, the difference in the area covered by tDCS is likely to cause the difference in the effects. tDCS electrodes of both sizes were evaluated by a 3D image and were found to cover PMd, but only the large electrode included SMA. We assume that tDCS over not only PMd but also SMA might be necessary to modulate the excitability of M1 and S1. Our results appear to differ from those of a recent tDCS study (Boros et al., 2008); anodal tDCS over PM decreased SICI and increased intracortical facilitation (ICF), whereas motor thresholds, single test-pulse MEPs, and input–output curves of MEPs remained stable in that study. However, our study findings are in part consistent with those of their study in that tDCS limited to PMd using the small electrode had no effect on single-pulse MEPs, although we did not evaluate SICI/ICF by paired-pulse TMS. Boros et al. (2008) used a large rectangular electrode (3 × 11 cm) to stimulate PM, and this large electrode may cover almost the entire PM area (PMd and PMv). This suggests that tDCS over PM using a large electrode could selectively modulate cortico-cortical excitability (SICI and ICF) but not corticospinal excitability (single-pulse MEPs and input–output curves). However, stimulating PMd as well as SMA would modulate corticospinal excitability as shown in our study.

High-frequency rTMS over PMd or SMA increased MEP amplitudes (Rizzo et al., 2003; Matsunaga et al., 2005; Raux et al., 2010). Conversely, low-frequency (1 Hz) rTMS decreased M1 excitability (Gerschlager et al., 2001; Münchau et al., 2002; Rizzo et al., 2003). However, Rizzo et al. (2003) demonstrated that high-frequency rTMS over PMd did not always increase M1 excitability. They found that 1- and 5-Hz rTMS over PMd had the opposite effect on ipsilateral M1 and that these effects alternated depending on the intensity of rTMS. Their results suggest that rTMS over PM can induce up- or down-regulation of neuronal circuits to M1 when a certain intensity and frequency are selected. Interestingly, Civardi et al. (2001) examined connections to M1 from frontal and medial cortices. Conditioning TMS 4–6 cm lateral to the motor hotspot reduced MEP amplitudes, whereas conditioning TMS 2 cm lateral increased these amplitudes. These results indicate that the motor association cortex has facilitatory and inhibitory neural connections to M1. Therefore, anodal tDCS over the motor association cortex using the large electrode might have increased mainly the inhibitory input to M1 in this study.

4.2. Possibility of current spread to M1

Nitsche et al. (2007) conducted a control experiment to rule out the possibility that tDCS-generated motor cortical excitability shifts were caused by transfer of current flow. They stimulated the hotspot of the abductor digiti minimi (ADM) with tDCS using a small electrode (3.5 cm^2) and showed selective enhancement of MEP amplitude after anodal tDCS of ADM but not FDI. This result suggests that tDCS-generated modulation of cortical excitability can be restricted to the area under the stimulation electrode.

Edges of both the small and large electrodes in the present study were close to, but did not cover, FDI hotspot. Moreover, our study showed the differential effects of the two sizes of electrode, even though these electrodes were similarly close to the hotspot. Thus, we conclude that the difference in the excitability of M1 and S1 was caused by the neuronal connectivity from the motor association cortex to the sensorimotor cortex and not merely by the transfer of current flow over the scalp.

4.3. Sensorimotor interaction by tDCS over the motor association cortex

SEPs of the parietal electrode in this study were enhanced by anodal tDCS compared with the cathodal condition when MEPs were inhibited, and the effect was opposite for cathodal tDCS. Thus, S1 excitability was modulated in the direction opposite to that of M1 excitability. Several studies of rTMS or tDCS over M1 suggested that the amplitudes of MEPs and SEPs did not change in the opposite direction (Enomoto et al., 2001; Tsuji and Rothwell, 2002; Matsunaga et al., 2004; Wolters et al., 2005; Dieckhöfer et al., 2006), except for a report which showed that inhibitory theta burst stimulation over M1 enhanced SEPs (Ishikawa et al., 2007). Alternatively, low-frequency or inhibitory rTMS over PM, which should inhibit MEPs as described in the previous section, increased SEP amplitudes (Hosono et al., 2008). The findings of Hosono et al. (2008) are compatible with those of our study in that S1 excitability is modulated in a direction opposite to that of M1 excitability. Thus, modulation of the motor association cortex might cause bidirectional modulation of MEPs and SEPs. We assume that these bidirectional modulations might be similar to mechanisms of SEP gating in which SEPs are inhibited when M1 is activated for movement. Parietal SEPs are known to be attenuated before spontaneous voluntary movement (Ogata et al., 2009) in which SMA as well as PMd can be important elements for sensorimotor circuits (Taniwaki et al., 2003). Anatomical and physiological studies in animals (Dum and Strick, 1991; Stepniewska et al., 2006) and in our fMRI study (Taniwaki et al., 2003) have shown neuronal connections between M1/S1 and PM/SMA. Abnormal sensory processing in the hand before movement has been reported in cases of dystonia (Murase et al., 2000; Kaji et al., 2005) in which PM hyperactivity during writing was reported in a study using $H_2{}^{15}O$ PET (Ceballos-Baumann et al., 1997). Taken together, these results indicate that a functional connectivity might exist between PM/SMA and S1 via direct cortico-cortical connections or via indirect intercortical pathways such as M1.

In conclusion, anodal tDCS over the motor association cortex decreased MEP and increased SEP amplitudes compared with cathodal tDCS, which would result from increases in inhibitory inputs from PM/SMA to M1 and from excitatory inputs to S1 and vice versa for cathodal tDCS. Therefore, tDCS is useful for modulating PM/SMA excitability and assessing the plastic functions of M1 and S1.

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References

Bäumer T, Schippling S, Kroeger J, Zittel S, Koch G, Thomalla G, et al. Inhibitory and facilitatory connectivity from ventral premotor to primary motor cortex in healthy humans at rest-A bifocal TMS study. Clin Neurophysiol 2009;120:1724–31.

Boros K, Poreisz C, Münchau A, Paulus W, Nitsche MA. Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. Eur J Neurosci 2008;27:1292–300.

Ceballos-Baumann AO, Sheean G, Passingham RE, Marsden CD, Brooks DJ. Botulinum toxin does not reverse the cortical dysfunction associated with writer's cramp. A PET study. Brain 1997;120:571–82.

Civardi C, Cantello R, Asselman P, Rothwell JC. Transcranial magnetic stimulation can be used to test connections to primary motor areas from frontal and medial cortex in humans. Neuroimage 2001;14:1444–53.

Dieckhöfer A, Waberski TD, Nitsche M, Paulus W, Buchner H, Gobbele H. Transcranial direct current stimulation applied over the somatosensory cortex

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