−615A/G	*n*	239	197		103	94		137	116		rs936462	–	
	A/A	1.000	1.000		1.000	1.000		1.000	1.000				
	A/G	0.000	0.000		0.000	0.000		0.000	0.000				
	G/G	0.000	0.000		0.000	0.000		0.000	0.000				
−603del/T	*n*	240	210		103	94		137	116		rs747303	–	Mitsuyasu et al. (1999)
	del/del	0.500	0.529	0.517	0.553	0.564	0.542	0.460	0.500	0.110			Okuyama et al. (2000),
	del/T	0.388	0.390		0.388	0.340		0.387	0.431				Mitsuyasu et al. (2001)
	T/T	0.113	0.081		0.058	0.096		0.153	0.069				
−600G/C	*n*	240	210		103	94		137	116		rs10902180	–	Mitsuyasu et al. (1999)
	G/G	0.963	0.990	0.107	0.981	1.000	0.499[e]	0.949	0.983	0.186[e]			
	G/C	0.038	0.010		0.019	0.000		0.051	0.017				
	C/C	0.000	0.000		0.000	0.000		0.000	0.000				
−598G/T	*n*	238	207		100	93		138	114		–	–	Present study
	G/G	1.000	0.995		1.000	0.989		1.000	1.000				
	G/T	0.000	0.005		0.000	0.011		0.000	0.000				
	T/T	0.000	0.000		0.000	0.000		0.000	0.000				
−597$(G)_{2-5}$	*n*	239	210		103	94		136	116		(rs3842250)	(IMS-JST186019)	Present study
	G2/G3	0.004	0.000	0.347[e]	0.010	0.000	0.616[g]	0.000	0.000	0.302[g]			
	G3/G3	0.151	0.124		0.097	0.117		0.191	0.129				
	G3/G4	0.481	0.438		0.553	0.479		0.426	0.405				
	G3/G5	0.004	0.010		0.010	0.011		0.000	0.009				
	G4/G4	0.360	0.419		0.330	0.372		0.382	0.457				
	G4/G5	0.000	0.010		0.000	0.021		0.000	0.000				
−521T/C	*n*	239	206		102	93		137	113		rs1800955	IMS-JST186020	Mitsuyasu et al. (1999),
	T/T	0.389	0.325	0.330	0.402	0.237	0.046	0.380	0.398	0.930			Okuyama et al. (2000)
	T/C	0.481	0.515		0.441	0.548		0.511	0.487				Mitsuyasu et al. (2001)
	C/C	0.130	0.160		0.157	0.215		0.109	0.115				
−376C/T	*n*	237	212		102	95		135	117		rs916455	IMS-JST186021	Mitsuyasu et al. (1999),
	C/C	0.814	0.835	0.808[f]	0.843	0.832	0.922[g]	0.793	0.838	0.452			Mitsuyasu et al. (2001)
	C/T	0.181	0.160		0.147	0.158		0.207	0.162				
	T/T	0.004	0.005		0.010	0.011		0.000	0.000				
−364A/G	*n*	237	212		102	95		135	117		rs916456	–	
	A/A	1.000	1.000		1.000	1.000		1.000	1.000				
	A/G	0.000	0.000		0.000	0.000		0.000	0.000				
	G/G	0.000	0.000		0.000	0.000		0.000	0.000				
−291C/T	*n*	234	214		102	95		132	119		rs916457	IMS-JST186022	Mitsuyasu et al. (1999),
	C/C	0.752	0.743	0.380	0.735	0.789	0.412[g]	0.765	0.706	0.501[g]			Mitsuyasu et al. (2001)
	C/T	0.209	0.238		0.206	0.189		0.212	0.277				
	T/T	0.038	0.019		0.059	0.021		0.023	0.017				
−234C/A	*n*	237	212		101	95		136	117		–	–	Present study
	C/C	0.996	1.000		1.000	1.000		0.993	1.000				
	C/A	0.004	0.000		0.000	0.000		0.007	0.000				
	A/A	0.000	0.000		0.000	0.000		0.000	0.000				
−128G/T	*n*	237	214		102	95		135	119		–	–	Mitsuyasu et al. (1999)
	G/G	0.996	1.000		1.000	1.000		0.993	1.000				
	G/T	0.004	0.000		0.000	0.000		0.007	0.000				
	T/T	0.000	0.000		0.000	0.000		0.000	0.000				
−11C/T	*n*	239	197		93	88		131	109		–	–	Cichon et al. (1995)
	C/C	1.000	1.000		1.000	1.000		1.000	1.000				
	C/T	0.000	0.000		0.000	0.000		0.000	0.000				
	T/T	0.000	0.000		0.000	0.000		0.000	0.000				

(continued on next page)

Table 2 (*continued*)

Polymorphism[a]		Genotype frequency									db SNP[c]	JSNP[d]	References
		All			Female			Male					
		Control	Schizophrenia	p[b]	Control	Schizophrenia	p	Control	Schizophrenia	p			
+31G/C	n	239	197		92	86		130	106		–	–	Cichon et al. (1995)
	G/G	1.000	1.000		1.000	1.000		1.000	1.000				
	G/C	0.000	0.000		0.000	0.000		0.000	0.000				
	C/C	0.000	0.000		0.000	0.000		0.000	0.000				
12-bp repeat	n	239	197		104	89		135	108		–	–	
(+64 to +87)	2/2	0.736	0.690	0.240	0.721	0.708	0.797[g]	0.748	0.676	0.205[g]			
	2/1	0.222	0.284		0.231	0.258		0.215	0.306				
	1/1	0.042	0.025		0.048	0.034		0.037	0.019				
21-bp deletion	n	239	197		102	88		134	106		–	–	
(+106 to +126)	+/+	1.000	1.000		1.000	1.000		1.000	1.000				
	+/–	0.000	0.000		0.000	0.000		0.000	0.000				
	–/–	0.000	0.000		0.000	0.000		0.000	0.000				
48-bp VNTR	n	237	212		102	95		135	117		–	–	Van Tol et al. (1992)
(+2689 to +2880)	4/4	0.696	0.736	0.618	0.716	0.726	0.507	0.681	0.750	0.170			
	4/2	0.186	0.160		0.167	0.158		0.200	0.164				
	4/5	0.055	0.047		0.059	0.063		0.052	0.034				
	4/3	0.013	0.014		0.029	0.000		0.000	0.026				
	4/6	0.017	0.009		0.000	0.011		0.030	0.009				
	4/7	0.013	0.005		0.000	0.000		0.022	0.009				
	2/2	0.004	0.009		0.010	0.021		0.000	0.000				
	5/5	0.008	0.005		0.000	0.011		0.015	0.000				
	3/3	0.000	0.005		0.000	0.011		0.000	0.000				
	5/2	0.000	0.005		0.000	0.000		0.000	0.009				
	5/3	0.004	0.000		0.010	0.000		0.000	0.000				
	5/6	0.004	0.000		0.010	0.000		0.000	0.000				

[a] Polymorphism names of each SNP or the number below names stand for nucleotide varitation and relative position to the first nucleotide of the initiation codon of reference sequence AC021663 (141798 = +1).

[b] p values of χ^2 test (with Yates' correction for 2×2 table) were not corrected for multiple testing. There was no statistical significance after correction. Detailed statistical method was described in the text.

[c] dbSNP, a database of single nucleotide polymorphisms at National Center for Biotechnology Information.

[d] JSNP, a database of common gene variations in the Japanese population (Hirakawa et al., 2002).

[e] del, insertion/deletion polymorphism.

[f] n, the number of subject genotyped at each polymorphism.

found significant differences – before correction for multiple hypothesis testing – in the distribution of both −713C/T ($p = 0.049$) and −521T/C ($p = 0.046$, Table 2). In the case of −713C/T, the minor allele frequency was very low (0.02 in female schizophrenic patients, 0 in controls). There were four heterozygous schizophrenic patients (no rare homozygotes) compared to zero in the female controls. The −521C allele was more frequent in the female schizophrenic patients than the female controls ($p = 0.034$, OR:1.58, 95% CI: 1.06–2.37). When comparing the OR for each genotype using genotype T/T as the referent in the female group, the OR for T/C was 2.11 (95% CI: 1.10–4.07) while the OR for C/C was 2.33 (95% CI: 1.01–5.38). If the −521C allele behaved as a dominant, the OR for the combined C/C and T/C female group would be 2.17 (95% CI: 1.17–4.04, $p = 0.021$) relative to the T/T female group. However, when either the Bonferroni correction, or a less conservative modified Bonferroni that accounts for LD (Li and Ji, 2005; Nyholt, 2004) was applied these results were no longer significant.

There were no significant differences between patients and controls in the male subgroup, even before multiple hypothesis correction (Table 2). Likewise, stepwise logistic regression analyses failed to detect any significant association between polymorphisms and schizophrenia.

Having failed to detect any influence of individual polymorphisms on risk of schizophrenia, we next sought to determine whether *DRD4* haplotypes might influence schizophrenia risk. Before using software to predict haplotypes it is efficient to first remove polymorphisms that are in strong LD with other polymorphisms. Accordingly, we determined the LD coefficient D' and the correlation r^2 between all pairs of 17 polymorphisms (Fig. 2 and Table 3).

The International HapMap Project (http://www.hapmap.org) includes data on only five DRD4 SNPs that are polymorphic in Japanese: rs3758653 (5′ flanking region), rs3889692 (exon 3), rs11246226, rs936465 and rs4331145 (3′ flanking region). These SNPs were analyzed by Haploview. Only one LD block was formed, comprising the three downstream SNPs. Two HapMap SNPs, rs3758653 (−906T/C in this study) and rs3889692 (not genotyped in this study) were not correlated with each other or the other four SNPs (r^2 values 0.024–0.061). These results indicate low LD across the *DRD4* gene.

We used our genotype data to analyze LD in the 4.4-kb region of the *DRD4* gene and select tag-markers using

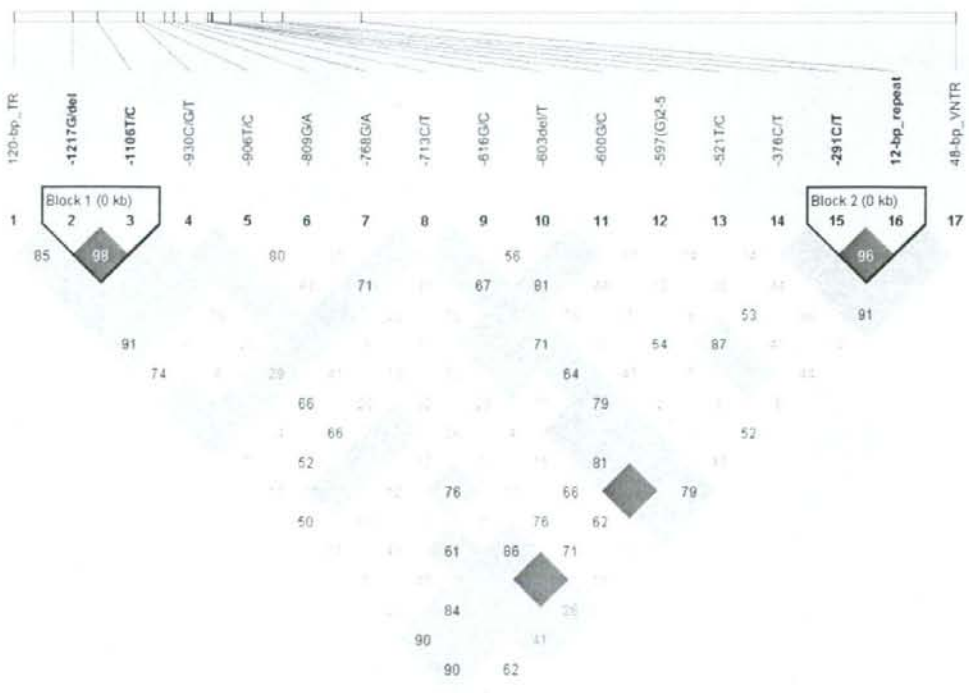

Fig. 2. LD coefficient D' representation of polymorphisms of the *DRD4* gene. The values in the boxes represent D' between pairs of markers. The LD display is from Haploview software. The boxes without values indicate complete LD ($D' = 1.0$). The dark grey boxes indicate strong LD. The light grey boxes indicate uninformative variant pairs. The white boxes indicate low LD. LD blocks were defined according to the algorithm in Haploview (Gabriel et al., 2002).

Table 3
Pairwise LD measure (r^2) of polymorphisms of the *DRD4*

	120-bp TR	−1217G/del	−1106T/C	−930C/G/T	−906T/C	−809G/A	−768G/A	−713C/T	−616G/C	−603del/T	−600G/C	−597$(G)_{2-5}$	−521T/C	−376C/T	−291C/T	12-bp repeat
−1217G/del	0.04															
−1106T/C	0.03	0.59														
−930C/G/T	0.05	0.00	0.00													
−906T/C	0.66	0.04	0.03	0.06												
−809G/A	0.46	0.00	0.01	0.06	0.62											
−768G/A	0.06	0.00	0.01	0.00	0.01	0.01										
−713C/T	0.03	0.00	0.00	0.00	0.03	0.02	0.00									
−616G/C	0.00	0.01	0.02	0.00	0.01	0.03	0.00	0.02								
−603del/T	0.01	0.02	0.02	0.00	0.01	0.01	0.00	0.01	0.28							
−600G/C	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.02	0.04						
−597$(G)_{2-5}$	0.02	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.14	0.00					
−521T/C	0.00	0.07	0.02	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02				
−376C/T	0.02	0.00	0.00	0.00	0.01	0.01	0.00	0.05	0.05	0.08	0.00	0.03	0.02			
−291C/T	0.43	0.02	0.02	0.06	0.39	0.28	0.07	0.00	0.00	0.00	0.00	0.03	0.02	0.02		
12-bp repeat	0.47	0.03	0.02	0.08	0.37	0.27	0.10	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.86	
48-bp VNTR	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.04	0.06	0.08	0.00	0.04	0.02	0.78	0.02	0.02

Pairwise LD measures (r^2) were calculated by Haploview software.

Tagger software in Haploview. Two small LD blocks were detected, one between −1217G/del and −1106T/C ($D' = 0.98$; $r^2 = 0.59$), the other between −291C/T and the 12-bp repeat ($D' = 0.96$; $r^2 = 0.86$). Other polymorphisms were only very weakly correlated, if at all ($r^2 < 0.80$). However, −376C/T and the 48-bp VNTR indicated relatively high correlation value ($r^2 = 0.78$). Based on this analysis 16 markers were selected for haplotype analysis. Thus, as a result of the low LD across the *DRD4* gene, we were only able to decrease the independent polymorphism number from 17 to 16 tag-markers.

The 48-bp VNTR polymorphism is in strong LD with −376C/T ($D' = 0.91$, $r^2 = 0.78$). However, it did not exhibit a high r^2 value with any other polymorphism in the region between the 120-bp TR and the 12-bp repeat of *DRD4* (Table 3). D' values between 120-bp TR and −906T/C ($D' = 0.91$) and −291C/T ($D' = 0.90$) were $\geqslant 0.90$. However, since corresponding r^2 values were less than 0.80, these polymorphisms could not be dropped based on our criteria for removing certain polymorphisms as described in Section 2.

Using 16 markers, a total of 136 haplotypes were estimated by PHASE. We compared the distribution of a total of 20 haplotypes with allele frequencies >0.01 between schizophrenics and controls in: (i) all subjects, (ii) female subgroup, and (iii) male subgroup. When the difference in haplotype frequencies was analyzed by the χ^2 test no significant differences were observed. Using 16 tag-markers, p values of sliding window haplotype analysis with window size 2 and 6 showed no statistically significant difference between schizophrenic patients and controls before adjustment for multiple hypothesis testing. Fig. 3 indicates the results of this analysis only for window sizes 2 and 3 (Fig. 3).

4. Discussion

In order to clarify the structure of genetic variation in the *DRD4* gene and to further explore potential genetic influences on schizophrenia, we genotyped 216 Japanese schizophrenics and 243 healthy controls at 27 polymorphic sites, including four novel SNPs.

Not surprisingly, we found the allele frequencies of some polymorphisms to be different in the Japanese population compared to European or other populations: −615A/G is polymorphic in Caucasians (Ronai et al., 2004), however, it was monomorphic in our study. The same phenomenon was observed with −364A/G, −11C/T and +31G/C (Cichon et al., 1995) and with a 21-bp deletion reported in a single individual suffering from obsessive-compulsive disorder and panic disorder (Cichon et al., 1995). Other polymorphisms (−1102A, −930T, −713T, −598T, −597$(G)_2$, −597$(G)_5$, −234A and −128T) had very low allele frequencies in the Japanese population (Table 2).

In order to assess the relationship between schizophrenia and *DRD4* polymorphisms, we carried out association analyses between Japanese schizophrenic patients and healthy controls. Univariate analyses indicated that none

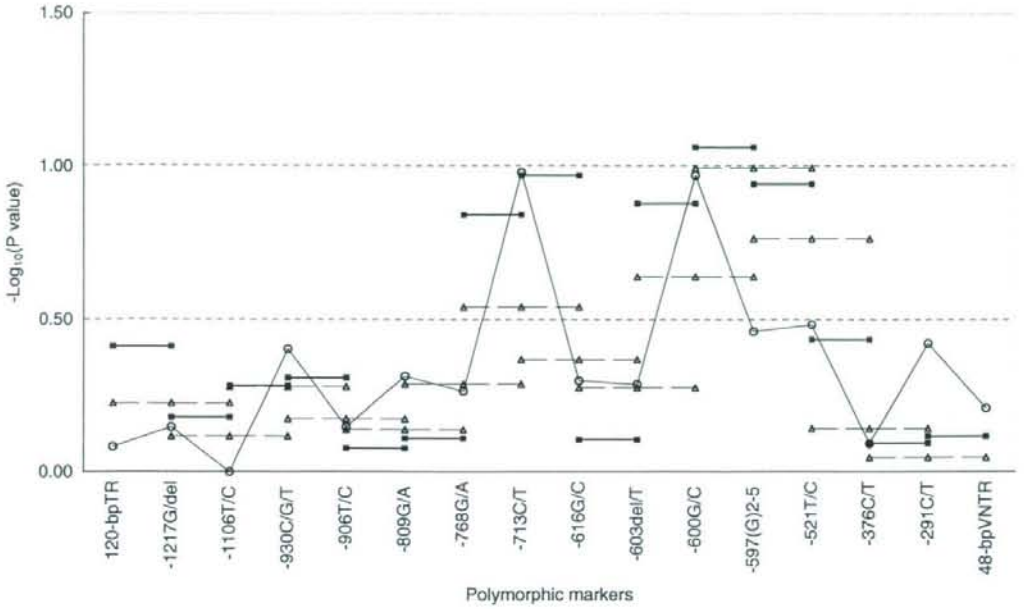

Fig. 3. Sliding window haplotype analysis of the *DRD4* gene. The *X*-axis displays each polymorphism analyzed in this study. The *Y*-axis shows $-\log_{10}(p$ value) of each marker and sliding window (window size 2 and 3) haplotype analysis. Open circles indicated the result of single marker analysis of each polymorphism (univariate analysis). Each line between two closed boxes indicates p value of 2-marker sliding window analysis. Each dashed line with three triangles indicates p value of 3-marker sliding window analysis.

of the markers was statistically significant after correction for multiple hypothesis testing.

There have been inconsistent reports regarding the −521T/C polymorphism in schizophrenia. Okuyama et al. reported that the T allele of this polymorphism reduces *DRD4* transcriptional efficiency by 40% compared with the C allele, and that, in the Japanese population, this marker is associated with schizophrenia (Okuyama et al., 1999). However, attempts to replicate these results in other populations such as Chinese and Caucasian have failed (Ambrosio et al., 2004; Jonsson et al., 2001; Xing et al., 2003). Based on these results one might speculate that there is heterogeneity in the genetics of schizophrenia. However, our negative findings regarding −521T/C in another Japanese population suggest that the result of Okuyama may reflect type I error.

We also carried out LD and haplotype analyses, however, the *DRD4* region is unusual both in terms of high SNP density and low LD. Consequently the potential power of haplotype based association methods is not much different from SNP based approaches. Only two LD blocks were formed in the *DRD4* region, each consisting of only two Polymorphisms, leaving most polymorphisms as independent variables. These results are consistent with other reports on the population genetic structure of *DRD4* (Wang et al., 2004). No statistically significant haplotype associations with schizophrenia were detected.

There are several limitations of this study that should be borne in mind. One concern is that the control population may not be perfectly matched with the schizophrenic population. Most of the male controls were Japanese Self Defense Forces personnel aged about 50 years old. There might be some characteristics of this population that differ from other healthy control populations. Ideally more detailed socioeconomic information should be collected to guide selection of a balanced control population, and for inclusion in a statistical model along with genetic variables. Also, in view of the effects of environmental factors on the development of schizophrenia, it is important to collect as much information as possible on environmental exposures.

In conclusion, we report in detail the structure of genetic variation across the *DRD4* gene in the Japanese population. LD analysis revealed two small LD blocks, however, the most notable pattern was low LD across most of the gene. Haplotype analysis using 16 tag-markers selected by LD block analysis revealed no associations with risk of schizophrenia. Despite the biological role of DRD4 in dopamine signaling, and reports of functional effects associated with polymorphisms such as the 48-bp repeat, this report contributes to the increasing body of literature suggesting that the gene does not contribute significantly to risk of schizophrenia.

Acknowledgments

We thank Ms. Akiko Tahara, Ms. Yoko Matsuo, Dr. Yukiko Koga, Mr. Leo Gotoh and Mr. Yuki Kobayashi for their technical help with the genotyping experiments.

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Repetitive transcranial magnetic stimulation alters optic flow perception

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Received 22 September 2006; accepted 24 September 2006

Optic flow, the visual motion radiating from the center to side or opposite directions, is used to control human locomotion. Low-frequency repetitive transcranial magnetic stimulation (0.9 Hz, 10 min) was applied to the primary visual cortex (V1) and the extrastriate area (V5/MT) of 12 healthy participants to study effects of repetitive transcranial magnetic stimulation on coherent optic flow perception. Cz stimulation was used as control. Participants were instructed to correctly identify focus for dots with coherent optic flow motion. Ratios of reaction times between V1 and Cz or between V5 and Cz 40 min after repetitive transcranial magnetic stimulation significantly increased. These results suggest the prolonged inhibitory effect of low-frequency repetitive transcranial magnetic stimulation on optic flow perception. Low-frequency repetitive transcranial magnetic stimulation is a useful tool for exploring visuospatial cognition. *NeuroReport* 18:229–233 © 2007 Lippincott Williams & Wilkins.

Keywords: coherent motion perception, optic flow, prolonged effect, repetitive transcranial magnetic stimulation, visual cortex

Introduction

Transcranial magnetic stimulation (TMS) is a noninvasive method used to stimulate the human visual cortex in behaving participants. Single TMS over the occipital cortex induces flash-like light sensation, known as 'phosphenes' [1]. The minimum TMS intensity required to elicit phosphenes is defined as the phosphene threshold (PT). PT was demonstrated to remain temporally stable among participants. Visual imagery tasks decrease PT [2], and PT is lower in methylenedioxymethamphetamine (MDMA) users [3] and migraineurs [4]. Therefore, PT has been suggested to serve as an index of visual cortex excitability [5,6].

Recently, repetitive TMS (rTMS) has been widely used to modulate excitability of the regional cerebral cortex. Previous studies showed that the frequency of rTMS was an important factor. In general, low-frequency (LF) stimuli around 1 Hz reduce cortical excitability, whereas high-frequency (HF) stimuli >5 Hz facilitate excitability. The amplitude of pattern-reversal visual-evoked potentials (VEPs) in the first block and its habituation over sequential blocks decreases after LF-rTMS over the primary visual cortex (V1) in normal individuals [7]. In contrast, HF-rTMS in blind people has been reported to increase reading speed of Braille characters [8]. Several LF-rTMS studies have been previously conducted to investigate functions of the visual cortex. Stimulation of the occipital lobe increases PT [4,6], impairs visual mental imagery [9] and alters contrast detection [10].

Visual information is first carried to V1, and is subsequently processed through parallel pathways consisting of dorsal (spatial vision) and ventral (object vision) streams [11]. The extrastriate area (V5/MT) plays an important role in the dorsal pathway, and contributes to motion perception. The direction of locomotion can be determined from information defined by the optic flow (OF): that of self-motion can be directly perceived from the 'focus of radial outflow' in the OF pattern [12]. V5/MT is also a key structure for perception of OF [13]. In patients with Alzheimer's disease and mild cognitive impairment, OF perception is selectively impaired [14]. Previous studies have shown that motion priming was abolished [15], and motion after-effect duration was reduced [16] with HF-rTMS over V5/MT; however, there have been no studies on effects of LF-rTMS on coherent OF perception. Therefore, we investigated motion perception of OF after LF-rTMS over V1 or V5.

Methods

Participants

Seventeen healthy, right-handed participants (10 men and seven women; age range, 20–40 years; mean age, 25.1 years) with normal or corrected-to-normal vision participated in the experiments. All participants gave informed consent to the studies. The study was approved by the Ethics Committee of Kyushu University, and conformed with the tenets of the Declaration of Helsinki.

Phosphene threshold

On the first day of the study, PT and optimal position of TMS were determined. Participants sat on a chair in a dark

room, wore a blindfold and a swimming cap with a grid parallel to the medio-sagittal line and the interaural line to capture the accurate repositioning of the coil on the following days. We delivered paired pulse (interstimulus interval, 71 ms) TMS using a Magstim Rapid stimulator (Magstim Co., Whitland, Carmarthenshire, Wales, UK, maximal output 1.6 T) and a figure-of-eight coil (inner diameter, 70 mm) to determine PT. PT was defined as the minimal intensity of the stimulator output to evoke uniform phosphenes in at least two of three consecutive trials.

After dark adaptation for 10 min, PT of the right V1 was measured, which was completed in about 30 min. Subsequent light exposure for 5 min was given to avoid changes of visual cortex excitability owing to prolonged sensory deprivation [17]. After readaptation to darkness, PT of the right V5 was measured. We chose the right hemisphere because of its predominance in motion perception, as seen in VEP or PET studies [18,19]. The order to determine optimal positions and PTs for V1 or V5 was counterbalanced between participants.

The coil was initially positioned 1 cm laterally and 3 cm above the inion for V1, and 5 cm laterally and 3 cm above the inion for V5. Two participants did not perceive phosphenes until 80% of the stimulator output; they were excluded from the following experiments. If phosphenes were perceived, their optimal position in 1-cm steps, and optimal direction with 45° steps from upward to rightward directions, was determined.

Evaluation of optic flow perception

To assess OF perception, we used a Windows PC (NEC, Tokyo, Japan) and the 'presentation' software (neurobehavioral systems). Participants sat 57 cm away from a 15-inch monitor (30 × 22°) in a dark room. On the monitor with a black background, some of the 400 white dots, used as stimulus, were concentrated or expanded from the focus, whereas others move randomly (Fig. 1). Focus was set at 5° left or right from the center of the monitor. Participants were instructed to correctly identify the locations of focus as soon as possible by using computer mouse buttons with their thumbs. Luminance of the white dots was set at 48 cd/m^2, whereas that of the background was set at 0.1 cd/m^2; therefore, the contrast level was 99.6%. Ratios of dots (RODs) with coherent movement of 'concentrating' or 'expanding' varied at 11 steps from 5 to 70% in a random order. Each step consisted of 20 trials (1 session=20 trials × 11 steps). An OF stimulus was shown for 750 ms with an interstimulus interval of 1250 ms. Speed of dot movement was 5°/s. One session was completed in 10 min. Rate of correct choice and reaction time (RT) for each ROD were recorded.

Experimental procedures

Each experiment consisted of three sessions before, immediately after and 40 min after rTMS. rTMS (applied at a rate of 0.9 Hz for 10 min at PT intensity) was focused to Cz (control condition), to the right V1 or V5, on separate days in a counterbalanced order. Participants practiced the motion perception task 1 day before the experiment and performed one practice session before measurement on each experimental day.

Data analysis

We determined the threshold of motion perception (TMP), which was defined as the ROD yielding 81.6% of correct responses according to the Weibull's function (Fig. 2) for each participant. TMPs and mean RTs of V1 or V5 stimulations were standardized against values obtained with Cz stimulation. Effects of rTMS on TMPs and mean RTs were evaluated using two-way repeated measures analysis of variance (ANOVA) with time (before, immediately after and 40 min after rTMS) and site of stimulation (V1/Cz, V5/Cz) as within-subject factors. A post-hoc analysis was performed using a multiple comparison with Bonferroni correction. A P value less than 0.05 was considered significant for all statistical analyses.

Results

Phosphene threshold

Fifteen of 17 participants perceived phosphenes, which tended to be on the contralateral side of rTMS, and most of them were white or light green. Two participants stimulated at V1 and four participants at V5 experienced moving phosphenes. Two of 15 participants conflicted with the schedule and one participant felt discomfort during rTMS; so, only the remaining 12 participants participated in the following rTMS experiments. Mean PTs were 58.9% for V1 and 57.5% for V5, respectively. Optimal direction of the handle of the magnetic coil was mostly rightward for V1, but rightward or oblique for V5.

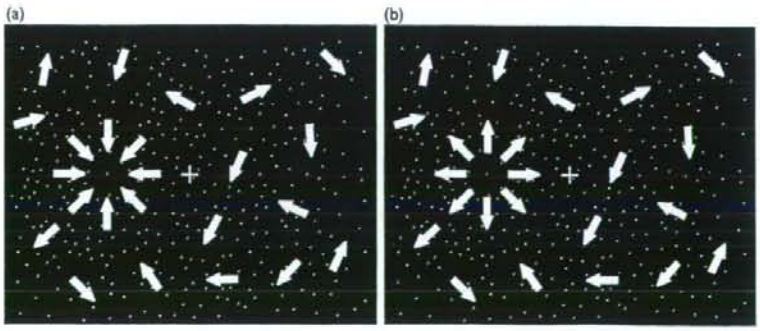

Fig. 1 Optic flow stimuli used in this study. Some of the 400 white dots used as stimulus were concentrated (a) or expanded (b) from the focus point. Participants were instructed to correctly identify locations of the focus that was left or right of the fixation point (cross).

Effect of repetitive transcranial magnetic stimulation on optic flow perception

Mean RTs at all levels of ROD and TMP for each stimulus site (V1/Cz, V5/Cz) are shown in Fig. 3. No significant temporal changes in RTs and TMPs were seen. We assumed that RTs could change mostly around TMP. Thus, we arbitrarily divided mean RTs into three subgroups: mean RTs around the threshold, subthreshold and suprathreshold (Fig. 4). Mean RT at threshold was calculated by averaging RTs at four ROD levels around TMP. Two-way ANOVA revealed a significant temporal increase in RT [$F(2,22)=4.943$; $P=0.017$], but neither a significant effect of stimulus site (V1/Cz vs. V5/Cz) nor a significant interaction between time and stimulus site was found. Multiple comparisons between time points revealed a significant decrease in RT between before rTMS and 40 min after rTMS ($P=0.023$). These results indicated that RTs for V1 and V5 stimulations increased 40 min after rTMS compared with Cz stimulation.

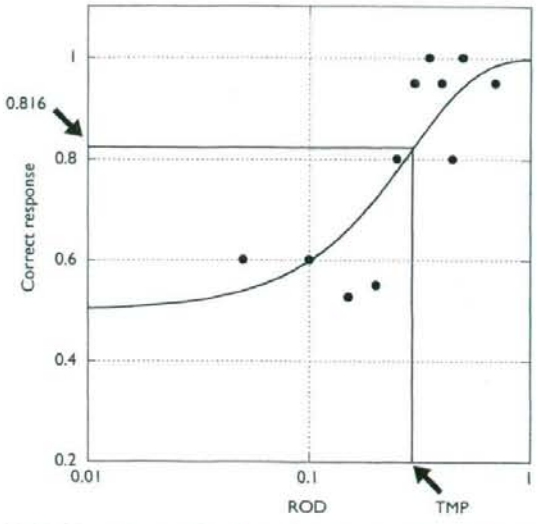

Fig. 2 Determination of the threshold of motion perception (TMP). TMP of a session (arrow) for each participant was defined as the ratio of dots (RODs) with coherent movement in stimuli yielding 81.6% of correct responses plotted according to the Weibull's function. The curve was fitted for 11 steps of ROD (filled dots). The correct response for each step was calculated for 20 trials.

Discussion

In this study, LF-rTMS over V1 or V5 inhibited OF perception, which lasted as long as 40 min as assessed by RT. We determined PT by paired TMS because of the difficulty in evoking phosphenes using single TMS in a preliminary study. Paired TMS reduced PTs as reported previously [1]. As we used rTMS with a single pulse, the intensity of rTMS was relatively low compared with paired pulse rTMS. Despite the subthreshold rTMS, we demonstrated an inhibitory effect of LF-rTMS on the visual cortex. This result was in good agreement with the results of previous studies showing that subthreshold LF-rTMS inhibited functions of the motor or visual cortex [6,20].

Significant differences were found in RTs but not in TMPs. This implied that RT might be more sensitive to cognitive changes than accuracy rate in rTMS studies [15]. So, how does RT really reflect motion perception? RT consists of visual perceptual and postperceptual (including motor response) processings; however, decision timing is not easy to specify. A recent magnetoencephalographic study [21] showed that accumulated activities in extrastriate areas identified the plausible timing of motion perception that was 150–200 ms before participants manually reacted to stimulation. Therefore, effects of LF-rTMS in our study may result from a decrease in rate or number of firing neurons in visual areas.

We also observed a significant inhibitory effect after 40 min rather than immediately after rTMS. In contrast, many rTMS studies revealed an immediate effect that lasted for a short period [7,20]. The mechanism of rTMS remains largely unclear, but the prolonged effect in our study may be related to long-term depression caused by synaptic changes or cell excitability changes [22].

OF is a stimulus related to motion perception and is important for locomotion or navigation [12]. A previous PET study [19] showed that V3 and other areas in addition to V5 and V1 were activated by OF. We demonstrated that rTMS

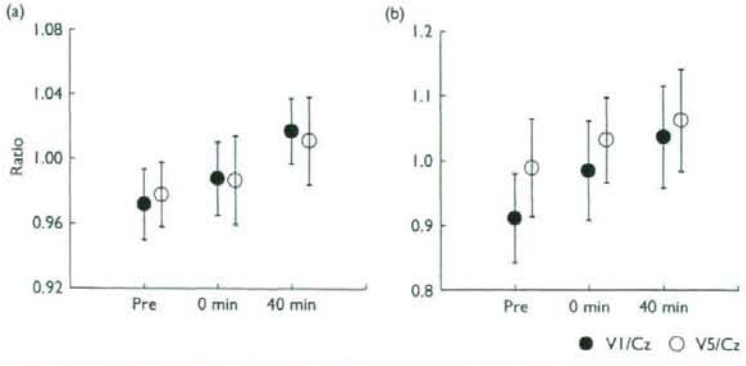

Fig. 3 Performance of motion perception: reaction time (RT) (a) and threshold of motion perception (TMP) (b). Mean RTs and TMPs of V1 or V5 stimulations were standardized against values for Cz stimulation. RTs and TMPs tended to be prolonged with time, but did not reach significant levels. Error bars indicate standard errors of means. Pre, before repetitive transcranial magnetic stimulation (rTMS); 0 min, immediately after rTMS; 40 min, 40 min after rTMS.

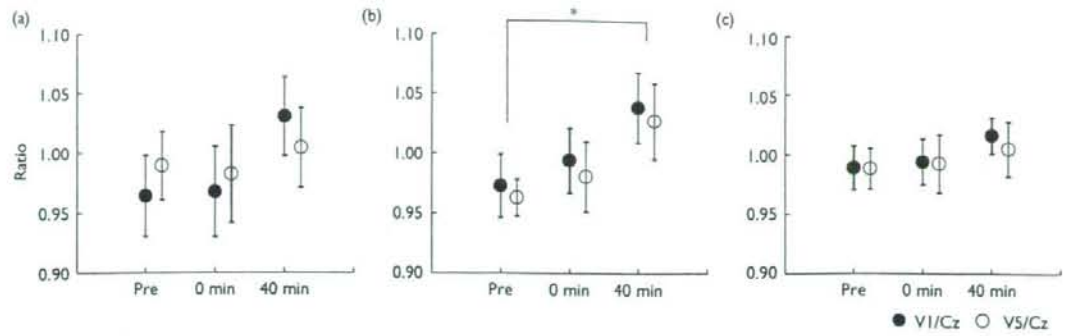

Fig. 4 Classification of reaction times into three groups: subthreshold (a), threshold (b) four steps around threshold of motion perception) and suprathreshold (c). Reaction times to the threshold level show clear changes compared with other groups, indicating a significant increase at 40 min after repetitive transcranial magnetic stimulation over V1 or V5. Error bars indicate standard errors of means. *Multiple comparisons between time points revealed a significant decrease in RT between before and 40 min after rTMS (P=0.023).

over V1 or V5 could modulate OF perception in similar manners, indicating that both V1 and V5 were important for OF perception. It is well known that visual information is processed step by step from V1 to V5/MT, and spreads to higher cortical areas. V1 and V5 stimulations may differentially affect OF perception. In fact, motion after-effect and visual motion priming were also disrupted by rTMS over V5 and not over V1 or posterior parietal cortices [15,16]. When single TMS was applied to V1 after V5, but not before V5, there was a marked decrease in quantity, and a change in quality of phosphenes elicited by V5 stimulation [23]. In addition, double-pulse TMS over V1 or V5/MT during a coherent motion task showed double dissociation in critical time windows, in which critical periods of V1 both preceded and followed that of V5/MT [24]. Although V5/MT obtains visual information through V1 feed-forward activity, back-projections from V5/MT to V1 are also critical for awareness of motion [23,24]. Thus, V1 and V5/MT differentially contribute to motion perception in a time-dependent manner. Although previous studies used single or paired TMS, LF-rTMS may result in modulation of motion perception through V1 as well as V5/MT.

OF is disturbed in patients with Alzheimer's disease and mild cognitive impairment [14], and VEPs to OF in Alzheimer's disease have also been reported to be impaired [25]. Subthreshold LF-rTMS over V1 or V5 in healthy participants can mimic visuospatial disturbance of such diseases as virtual brain lesions, as shown in this study.

Conclusion

LF-rTMS over V1 or V5 altered OF perception until 40 min after stimulation. This prolonged effect probably resulted from long-term depression. Therefore, LF-rTMS can be a useful tool for exploring visuospatial cognition.

Acknowledgements

This study was supported in part by a Grant-in-Aid for the 21st Century COE program and Grant-in-Aids for Scientists nos 16390253 and 16200005 from the Ministry of Education, Culture, Sports, Science and Technology, Japan. All authors declare that they had no conflicts of interest.

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Journal of the Neurological Sciences

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A distinct subgroup of chronic inflammatory demyelinating polyneuropathy with CNS demyelination and a favorable response to immunotherapy

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Received 7 September 2006; received in revised form 18 December 2006; accepted 3 January 2007
Available online 15 February 2007

Abstract

To explore subclinical central nervous system (CNS) involvement in chronic inflammatory demyelinating polyneuropathy (CIDP), we recorded somatosensory evoked potentials (SEPs) and motor evoked potentials (MEPs) using transcranial magnetic stimulation, to measure central sensory conduction time (CSCT) and central motor conduction time (CMCT) and examined brain and spinal cord MRI in patients with probable CIDP based on the American Academy of Neurology AIDS Task Force criteria. Eighteen patients with probable CIDP (12 males and 6 females; mean age at examination±SD, 45.8±17.0 years; range, 17–72) were included in the study. Of the 13 patients who underwent SEPs, one had prolonged CSCT (8%) and of the 13 who underwent MEPs, four had abnormal CMCT (31%). Cranial MRI revealed five of 18 patients had abnormal scans, only one of which showed multiple ovoid periventricular lesions suggestive of demyelination while none showed any intramedullary lesion on spinal cord MRI. Thus, 6 of the 18 patients were considered to have subclinical demyelinative CNS involvement which had lower disability on Global Neurological Disability Score (GNDS) ($p=0.0061$), a male preponderance (0.0537) and a larger compound muscle action potential (CMAP) amplitude in the median nerve ($p=0.005$) than those without. The decrease of GNDS with immunologic therapies was nearly significant in the former ($p=0.0556$) but not in the latter. The results of the present study suggest that subclinical CNS involvement in CIDP is not uncommon in Japanese patients and that CIDP with subclinical CNS involvement is more demyelinative thus responsive to immunotherapies while those without have more axonal damage and less responsive to immunotherapies.

Keywords: Chronic inflammatory demyelinating polyneuropathy; Motor evoked potentials; Somatosensory evoked potentials; Central nervous system

1. Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is considered to be an autoimmune disorder of the peripheral nervous system (PNS). Although peripheral myelin is targeted by an autoimmune attack, central nervous system (CNS) involvement has been suggested in a fraction of CIDP patients with the presence of subclinical electrophysiologic and magnetic resonance imaging (MRI) abnormalities.

In two large series, CNS involvement was clinically observed in 5% and 8% of patients, respectively [1,2]. In the electrophysiological study by Ormerod et al. [3], six of 18 patients (33%) had unilateral or bilateral abnormalities in central motor conduction time (CMCT) on motor evoked potentials (MEPs). On brain MRI, a third to a half of CIDP patients have been reported to have brain lesions [3–6] whereas demyelinating lesions with typical appearance of multiple sclerosis (MS) are uncommon; for example, two of

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doi:10.1016/j.jns.2007.01.004

26 patients (8%) in the series of Hawke et al. [6]. As non-specific brain lesions are frequently encountered on MRI, the electrophysiological methods may be more suitable for detecting demyelinative CNS lesions in CIDP. Therefore, the present study was undertaken firstly to explore subclinical CNS involvement in CIDP patients without clinically overt CNS signs, by electrophysiological methods such as MEPs and somatosensory evoked potentials (SEPs), and by brain and spinal cord MRI. Moreover, it was recently shown that in CIDP patients, treatment response to intravenous immunoglobulin (IVIg) administration was in part determined by degree of peripheral axonal involvement with poor response in those with greater axonal damage [7]. However, it is unknown whether CIDP patients with subclinical CNS involvement respond to immunotherapies well or not. Thus, secondly, we aimed to clarify the difference between patients with subclinical CNS involvement and those without.

2. Subjects and methods

2.1. Subjects

Eighteen consecutive patients with probable CIDP (12 males and six females; mean age at examination±SD, 45.8±7.0 years; range, 17–72) based on the criteria of the American Academy of Neurology AIDS Task Force were included [8]. The demographic features of the patients are described in Table 1. The mean age of onset was 40.5±20.7 years (mean±SD; range 17–72 years). The duration of disease ranged from 2 months to 36 years (mean±SD=5.8±10.9 years). Seven presented with weakness while eleven with combined weakness and sensory impairment. At some stage in the clinical course, all of the patients showed distal limb weakness while sensory disturbance was present in 15. None of the patients had clinical signs of CNS involvement. The clinical courses were chronic progressive in 10 patients, relapsing–remitting in three, and monophasic in five. For the assessment of neuropathy, the Global Neurological Disability Score (GNDS) was used to initially assess the motor neurological disability, sensory loss and areflexia on a scale of 1 to 15 [9]. The GNDS scores before treatment were 12.5±3.0 (mean±SD, range: 6–15). On nerve conduction studies, motor nerve conduction velocities (MCV) were all reduced in all patients in at least more than one nerve. Sensory nerve conduction velocities (SCV) were normal in 5/18, unevoked in 7/18 and reduced in 6/18 in the median nerve and normal in 4/18, unevoked in 8/18 and reduced in 4/18 in the sural nerve. Protein levels in the cerebrospinal fluid (CSF) were elevated in 13 of 17 examined (>40 mg/dl) while none had CSF pleocytosis. All but one patient were subjected to immunotherapies and a 2-point decrease in the GNDS score was considered to be effective. Seven of 17 patients (41.1%) responded to immunotherapies; high-dose corticosteroids (prednisolone 40–60 mg/day with gradual taper) were effective in two of four patients who received it, IVIg was effective in three of five patients, and plasma exchange (PE) was effective in two of five. Three patients who received PE and IVIg showed no

Table 1
Demographic features of patients with CIDP

Patient No.	Sex	Age at onset (year)	Age at exam. (year)	Duration (months or years)	GNDS at peak	CSF cell/protein (/μl, mg/dl)	Clinical course	Response to immunotherapies	Clinical symptoms		Evoked potentials			
									Predominant symptoms	Symmetrical involvement	MEP abnormality		SEP abnormality	
											CNS	PNS	CNS	PNS
1	M	58	65	8y	15	3/217	R	IVIg: +	motor>sensory	+	−	+	ND	
2	M	28	28	1y	11	5/100	CP	IVIg: +	motor>>sensory	+	+	+	−	+
3	F	58	59	4m	15	0/33	CP	IVIg: +	motor>sensory	+	−	+	−	−
4	M	17	17	1y	15	1/58	CP	PE: −	motor	+	−	+	ND	
5	M	47	52	5y	15	1/34	CP	IVIg, PE: −	motor>sensory	+	−	+	ND	
6	F	51	52	1y	11	3/178	CP	PE: −	motor>sensory	+	−	+	−	−
7	M	31	32	11m	9	0/320	CP	IVIg, PE: −	motor>sensory	+	−	+	−	−
8	F	48	52	5y	15	1/55	CP	PE: +	motor>sensory	−	−	+	−	−
9	M	12	18	6y	15	0/266	CP	PE: −	motor>sensory	+	−	−	ND	
10	M	11	45	34y	9	0/67	R	CS: +	motor	+	−	+	+	−
11	M	66	67	1y	15	3/290	CP	CS: −	motor>sensory	+	ND		−	−
12	M	36	42	6y	6	3/62	R	IVIg, PE: −	motor>sensory	+	+	+	−	+
13	M	69	69	2m	13	1/57	M	PE: +	motor>sensory	+	+	+	ND	
14	F	26	26	2m	11	1/157	M	CS: +	motor	+	ND		−	+
15	M	46	46	2m	15	2/127	M	CS: −	motor>sensory	+	ND		−	−
16	M	1	37	36y	8	2/27	CP	IVIg: −	motor>sensory	+	+	+	−	+
17	M	46	46	2m	13	ND	CP	ND	motor=sensory	+	ND		−	+
18	F	72	72	2m	15	1/28	CP	IVIg: −	motor>sensory	−	ND		−	−

M: male; F: female; m: months; y: years; CSF: cerebrospinal fluid; R: relapsing–remitting; M: monophasic; CP: chronic progressive; ND: not done; GNDS: Global Neurological Disability Score.
Response to immunotherapies given.
IVIg: intravenous immunoglobulin (IVIg), PE: plasma exchange (PE), CS: corticosteroids.
+: effective (2 or >2-point decrease in GNDS scores), −: no change (<2-point decrease in GNDS scores).

significant improvement. One patient did not undergo any treatment.

2.2. Somatosensory evoked potential recording

The SEPs were obtained by stimulating the median nerve at the wrist and the posterior tibial nerve at the ankle with frequencies of 5 Hz and 2 Hz respectively [10]. Recording electrodes were placed over Erb's point, seventh cervical vertebra, and C3′ or C4′ over the somatosensory cortex; for the lower extremities, the electrodes were placed over the 12th thoracic vertebra and Cz′. Fz was used as the reference of all electrodes. The amplifier used was a Neuropack 8 (Nihon kohden) with a bandpass of 5–2000 Hz and averaged at 500 for the uppers and 350 for the lowers. At least two trials were superimposed to establish reproducibility. The peak latencies of the responses were measured: N9 (Erb), N13 (C7) and N20 (sensory cortex) for median nerve SEPs and N20 (Th12) and P37 (sensory cortex) for tibial nerve SEPs. Central sensory conduction time (CSCT) was calculated as N20–N13 for the upper extremities while P37–N20 for the lower extremities. The normal values for SEPs in our laboratory are as follows: for the upper extremities, for median nerve stimulation, the mean for N13–N20 is 5.89 with an upper limit of 7.33 ms, while for the lower extremities, with posterior tibial nerve stimulation, the mean for N20–P37 is 16.88 ms with an upper limit of 21.83 ms, while for peroneal nerve stimulation, the mean for N13–P28 is 14.5 ms with an upper limit of 20.08 ms [10]. Latencies exceeding the mean+3SD from the established normal values for SEPs were considered abnormal.

2.3. Motor evoked potential recordings

Magnetic stimuli were applied to the motor cortex and the seventh cervical vertebra using an eight-shaped coil for the upper extremities while a double cone coil was used for stimulating the motor cortex for the lower extremities, and the lumbar root (L4) was elicited by the eight-shaped coil [11]. The target muscles were the abductor pollicis brevis for the hands and the abductor hallucis for the legs. The stimulator used was an SMN-1200 (Nihon kohden) with a stimulus intensity of 65% of stimulator output for the upper extremity and lumbar, and 90% was used for the vertex. The amplifier was a Neuropack 8 (Nihon kohden) and the bandpass of 50–3000 Hz. We assessed MEP latencies and amplitudes (qualitatively) and calculated the central motor conduction time (CMCT): CMCT=CML−PML (CML: cortical motor latency; PML: peripheral motor latency). Normal values: the normal mean central conduction used in our laboratory for the thenar muscle central conduction is 8.61 ms with an upper limit of 10.67 ms while for the plantar muscle the mean central conduction time is 16.94 ms with an upper limit of 21.04 ms [10]. Latencies exceeding the mean+3SD from the established normal values for MEPs were considered abnormal.

2.4. Magnetic resonance imaging

MRI was performed using 1.5 T units, Magnetom Vision and Symphony (Siemens Medical Systems, Erlangen, Germany) as described previously [12]. The typical imaging parameters for brain MRI were: axial T2-weighted turbo spin-echo imaging using TR/TE=2800/90 ms, flip angle=180°; axial turbo-FLAIR imaging using TI/TR/TE=2200/9000/110 ms, flip angle=180°; and sagittal and axial precontrast and axial and coronal postcontrast T1-weighted spin-echo imaging using TR/TE range=400–460/12–17 ms, flip angle range=80–90°. One excitation, with a matrix of 256×256, a slice thickness of 5 mm, and a slice gap of 2.5 mm was used for all brain studies. Gadopentetate dimeglumine at 0.1 mmol/kg body weight was administered intravenously for contrast-enhanced studies. The typical imaging parameters for the spinal cord were as follows: sagittal T2-weighted turbo spin-echo imaging using TR/TE range=2500–2800/90–116 ms, flip angle=180°, number of excitations=3–4; sagittal T1-weighted spin-echo imaging using TR/TE range=400–440/11–12 ms, flip angle range=90–170°, number of excitations=2–3; axial T2-weighted turbo spin-echo imaging using TR/TE range=3200–5360/99–116 ms, flip angle=180°, number of excitations=3–4; axial T1-weighted spin-echo imaging using TR/TE range=400–440/12 ms, flip angle range=90–170°, number of excitations=2. For sagittal imaging, a matrix of 256×256 or 512×512, a slice thickness of 4 mm and a slice gap of 0.4 mm were used, and for axial imaging, a matrix of 256×256 or 512×512, a slice thickness of 5 mm, and a slice gap range of 1.5–5 mm were used. Both brain and spinal cord MRI's were taken at the time of illness and were independently evaluated by two of the authors, one of whom (T. Yoshiura) is a neuroradiologist who was unaware of the diagnoses.

2.5. Statistical analysis

Statistical analyses were performed using the Mann–Whitney U test to determine significant differences in age at onset, duration of disease and CSF protein levels between the two groups and the differences in GNDS scores between those with CNS involvement and those without and between before and after treatment. Fisher's exact probability test was used for sex ratio and clinical course. The p values of <0.05 were considered to be significant.

3. Results

3.1. Electrophysiological findings

Five of the 13 patients who underwent SEPs had peripheral nerve involvement, one (Patient No. 10 in Table 1) having prolonged CSCT (8%). This patient had bilateral lower extremity involvement (peroneals) with CSCTs of 22.72 ms on the right and 23.52 ms on the left. Another patient (Patient No. 15) had unevoked N13 but prolonged N20 on median

nerve SEPs bilaterally and was not regarded as having definite CNS involvement, though the possibility of CNS involvement was not fully ruled out. Of the 52 limbs examined, 12 (23%) showed prolonged latencies (compared with the normal values previously mentioned) for Erb (N9), and the seventh cervical vertebrae (N13) for the upper extremities and fourth lumbar vertebrae (N17) and twelfth thoracic vertebrae (N20) for the lower extremities with posterior tibial nerve stimulation. Of the 13 patients who underwent MEP, 12 showed peripheral involvement and four had abnormal CMCT (31%) (Table 1). Three of these four patients had unilateral involvement (left upper extremity with a CMCT of 10.8 ms, right lower extremity with a CMCT of 23.5 ms and left lower extremity with a CMCT of 39.6 ms) while one patient had bilateral lower extremity involvement with CMCT of 26.6 ms on the right and 28.4 ms on the left. Five additional patients showed unevoked MEPs at cervical or lumbar stimulation but prolonged latency of MEPs at cortical stimulation, and were not considered to have definite CNS involvement, though the possibility of CNS involvement was not completely excluded. Of the 52 limbs examined, 31 (60%) showed prolonged latencies with Erb, cervical or lumbar stimulation as compared with normal values.

3.2. Magnetic resonance imaging findings

On brain MRI, five of 18 patients had an abnormal MRI scan; of these, four were aged 50 years or older. One patient (Patient No. 13 in Table 1, 69 years old) with abnormal CMCT, showed punctate T2 prolonged lesions in the cerebral white matter on MRI, which were considered to be non-specific. In the other three patients without CMCT or CSCT abnormalities, one (Patient No. 3, 59 years old) showed T2 prolonged lesions in the right putamen and caudate nucleus suggestive of old small infarcts while the other two (Patients No. 6, 52 years old and No. 8, 52 years old) had tiny spots of T2 prolonged lesions in the white matter on cranial MRI, which were also considered non-specific. Patient No. 17, a 46-year-old male, on fluid-attenuated inversion recovery (FLAIR) imaging, showed multiple ovoid lesions of increased signal intensity in the corpus callosum bilaterally, and in the left parietal and occipital lobes which appeared MS-like (Fig. 1).

3.3. Clinical characteristics of patients with subclinical CNS involvement

For comparison of clinical features, we divided the patients by electrophysiologic and MRI findings into two groups, those with and those without subclinical CNS involvement; patients were regarded as having subclinical

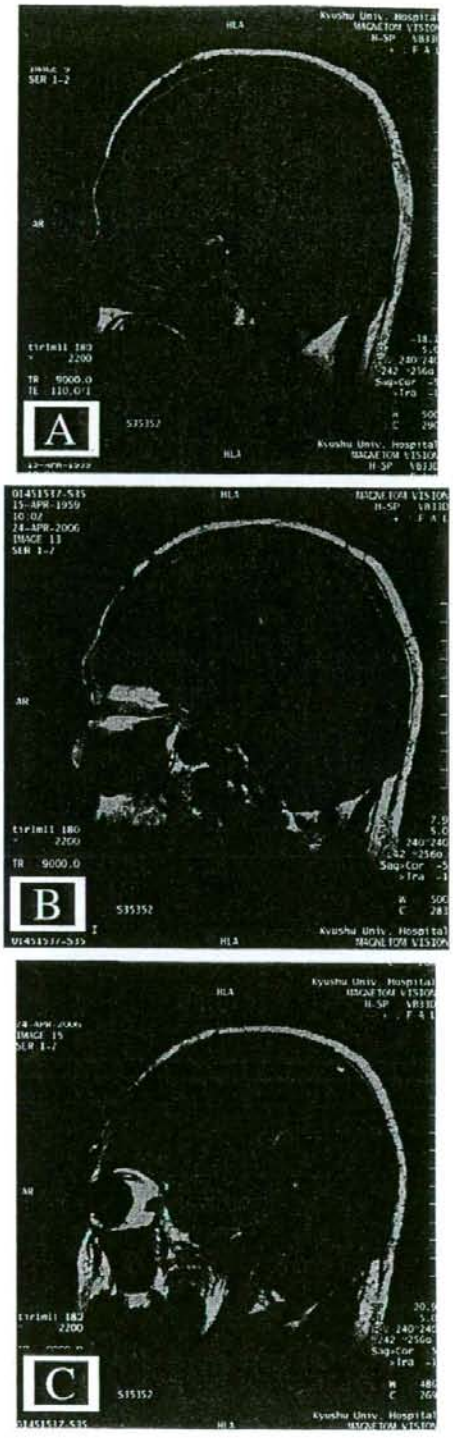

Fig. 1. Brain MRI of Patient No. 17. Sagittal fluid-attenuated inversion recovery (FLAIR) images (TR=9000 ms, TE=110 ms) of a 46-year-old male demonstrating multiple ovoid lesions of increased signal intensity in the corpus callosum, bilaterally, and in the left parietal and occipital lobes which appeared MS-like. [A] to [C]: right to left side of the patient.

demyelinative CNS involvement if they had prolonged CMCT or CSCT or MRI lesions suggestive of CNS demyelination (MS-like ovoid lesions). Based on the electrophysiological and MRI findings, six were considered to have subclinical demyelinative CNS involvement (Patient Nos. 2, 10, 12, 13, 16 and 17) and the other 11 were not. As shown in Table 2, the patients with subclinical CNS involvement showed a tendency of male preponderance ($p=0.0537$) and significantly lower GNDS scores before treatment ($p=0.0061$), as compared with those without CNS involvement. Most patients (83%) without CNS involvement showed a chronic progressive course while half of those with subclinical CNS involvement had either a relapsing–remitting or a monophasic course. In addition, the patients with subclinical CNS involvement had lower CSF protein levels than those without, but this was not statistically significant. In the peripheral nerve conduction study, CMAP amplitude in the median nerve was significantly larger in the patients with subclinical CNS involvement than those without ($p=0.005$). In addition, in half of CIDP patients without CNS involvement, tibial nerve CMAPs were unevoked while only one of six CIDP with subclinical CNS involvement were. Patients with subclinical CNS involvement showed a nearly significant decrease of GNDS scores after immunotherapy ($p=0.0556$) while the decrease of GNDS scores was not significant in those without CNS involvement (Table 2). GNDS scores after treatment were also significantly lower in the patients with subclinical CNS involvement than in those without ($p=0.0365$).

Table 2
Comparison of clinical findings between patients with subclinical CNS involvement by EP or MRI and those without

	CIDP with subclinical CNS involvement by EP or MRI ($n=6$)	CIDP without CNS involvement ($n=12$)
Male:female	6:0	6:6
Age at onset (mean±SD, years)	31.8±24.5	44.4±19.1
Duration of disease (range, median)	2m–34y 3.5y	2m–36y 1y
Clinical course/onset		
Chronic progressive	3 (50%)	10 (83%)
Relapsing–remitting	2	1
Monophasic	1	1
GNDS before treatment	9.4±2.7*	13.8±2.2
GNDS after treatment	5.8±1.8*	11.1±4.6
CSF protein	62.6±26.1	146.9±107.5
Median nerve		
MCV, m/s	34.7±13.5	29.8±14.2
DL, ms	6.6±3.0	7.6±3.8
CMAP, mV	15.4±7.0*	5.8±3.2
Unevoked	0/6	0/12
Tibial nerve		
MCV, m/s	35.7±5.2	29.5±10.4
DL, ms	5.6±2.5	11.6±6.9
CMAP, mV	5.9±4.7	4.3±6.2
Unevoked	1/6	6/12

GNDS: Global Neurological Disability Score; CSF: cerebrospinal fluid; MCV: motor nerve conduction velocity; DL: distal latency; CMAP: compound muscle action potential. One patient from the CNS involvement group declined to have a lumbar puncture and also did not receive any immunotherapeutic intervention.
* $p<0.05$.

4. Discussion

The present study revealed subclinical CNS involvement suggestive of demyelination in Japanese patients with CIDP by combined electrophysiological and neuroimaging studies. We further described the distinct clinical features between those with and those without subclinical CNS involvement; CIDP patients with subclinical CNS involvement had lower disability and a more favorable response to immunological treatment than those without.

Although the frequent occurrence of peripheral conduction abnormalities in CIDP patients may well decrease the detection rate of CNS abnormality by EP, in the present study, about 30% of the CIDP patients demonstrated prolongation in either CMCT or CSCT, suggesting the presence of simultaneous CNS demyelination. The frequency of CNS abnormalities on EP in the present study is compatible with that reported in the previous studies [3,13]. However, though Mendell et al. [4] reported that approximately 40% of CIDP patients had MS-like periventricular, subcortical and brainstem lesions on MRI in their selected patient series, MS-like periventricular ovoid lesions were seen in only one patient in our series. Our findings are in accord with the report of Feasby et al. [5] that typical MS-like lesions are uncommon in CIDP. Our results are also compatible with those of Pakalnis et al. [13], who concluded that with a combined EP and MRI study, EP is more sensitive than MRI in detecting CNS demyelination in CIDP. Therefore, we consider that subclinical CNS involvement is not infrequent on EPs while typical MS-like lesions suggestive of demyelination on MRI are exceptional in Japanese patients. It is suggested that a combined peripheral and central nervous system demyelination is not rare, but the nature and mechanism of CNS demyelination in CIDP is probably distinct from those in MS.

Neither characteristic clinical features nor response to immunotherapies has yet to be described in CIDP patients with subclinical CNS involvement. In our series, although the number of patients is limited, it was shown that those with subclinical CNS involvement had a milder disease, and chronic progressive disease was less frequent compared with those without CNS involvement, and showed a favorable response to immunotherapies. Although therapeutic modalities were not controlled in the present study, it has been shown that IVIg, PE and corticosteroids have essentially the same efficacy in CIDP [14,15]. In previous studies, axonal loss, as shown by a decrease in CMAP or nerve biopsy, was correlated with a significantly poor response to immunotherapies such as IVIg [1,7]. In the present study, CMAP amplitudes in median nerve were significantly larger in CIDP patients with subclinical CNS involvement than those

without, while in the tibial nerve, unevoked response was more frequent in the latter than in the former. These findings, although seen in a small number of patients and a generalized conclusion should be drawn with caution, are still suggestive that CIDP patients without CNS involvement suffer more severe axonal pathology than those with subclinical CNS involvement. The high frequency of chronic progressive course in those without CNS involvement may also contribute to more severe axonal damage [7]. Such difference in pathology may in part explain the difference in treatment response between the two subgroups; CIDP with subclinical CNS involvement is more demyelinative and thus responsive to immunotherapies while CIDP without CNS involvement is more axonal and less responsive to such therapies. Fee and Fleming [16] reported that IVIg resolved CNS demyelinative lesions in a case of CIDP. Thus, subclinical CNS lesions are also likely to be caused by the same immune mechanism as that involved in PNS demyelination in this condition.

The presence of a combined central and peripheral inflammatory demyelinating neuropathy has long been proposed in the literature [3,4,6]. The results of our study support such a notion. Whether CIDP with subclinical CNS demyelination is distinct from CIDP without CNS involvement in etiology and mechanism remains to be elucidated, the combined use of MEP/SEP and brain MRI may help identify a subgroup of CIDP patients with combined central and peripheral demyelination. Future immunological and pathological studies in a larger group of patients and controlled therapeutic trials on this specific subgroup of CIDP patients are called for to further clarify the mechanisms behind this debilitating disease of the human nervous system.

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Clinical Neurophysiology 118 (2007) 1198–1203

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Altered soleus responses to magnetic stimulation in pure cerebellar ataxia ☆

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Accepted 7 March 2007
Available online 23 April 2007

Abstract

Objective: Transcranial magnetic stimulation (TMS) over the leg motor area elicits a soleus primary response (SPR) and a soleus late response (SLR). We evaluated the influence of the cerebellofugal pathway on the SPR and SLR in patients with 'pure' cerebellar ataxia.
Methods: SPRs and SLRs were recorded from 11 healthy subjects and 9 patients with 'pure' cerebellar cortical degeneration; 5 with spinocerebellar ataxia type 6 (SCA6), and 4 with late cortical cerebellar ataxia (LCCA). In addition, three patients with localized cerebellar lesions were tested.
Results: The SPR latency was significantly longer in patients than in controls, but primary responses in the tibialis anterior muscle were normal. The frequency of abnormal SLR was 38.9% in the supine position and 83.3% in the standing position. Two out of three patients with localized cerebellar lesions also showed abnormal SLR.
Conclusions: Altered SPRs in patients may result from a dysfunction of the primary motor cortex caused by crossed cerebello-cerebral diaschisis. In addition, our results suggest that 'pure' cerebellar degeneration involves the mechanism responsible for evoking SLR which is related to the control of posture.
Significance: SLR can be a useful neurophysiological parameter for evaluating cerebellofugal function.

Keywords: Soleus primary response (SPR); Soleus late response (SLR); Transcranial magnetic stimulation (TMS); Cerebellar degeneration; Posture

1. Introduction

Transcranial magnetic stimulation (TMS) of the motor cortex elicits signals that are transmitted by the corticospinal tract to the four limbs to evoke primary motor evoked potentials (MEPs). In the soleus (SOL) muscle, TMS elicits a primary response with a latency of about 30 ms followed by a late response with a latency of about 90 ms (Dimitrijević et al., 1992; Ertekin et al., 1995; Holmgren et al., 1990; Sammut et al., 1995; Valls-Solé et al., 1994). The soleus primary response (SPR) has been considered to be the direct activation of the corticospinal pathway (Lavoie et al., 1995; Wochnik-Dyjas et al., 1998), and is enhanced by standing or voluntary contraction (Ackermann et al., 1991; Valls-Solé et al., 1994). The soleus late response (SLR), on the other hand, is more prominent when the tibialis anterior (TA) is activated weakly (Ertekin et al., 1995;

☆ Disclosure: The authors have no conflict of interest.

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1388-2457/$32.00
doi:10.1016/j.clinph.2007.03.006

Sammut et al., 1995; Suga et al., 2001), and is sometimes large even when the primary SOL response is small (Ertekin et al., 1995). In addition, the SLR is enhanced when standing is made difficult and is diminished by contracting the SOL (Ertekin et al., 1995; Sammut et al., 1995; Suga et al., 2001) or when the ankle is immobilized (Sammut et al., 1995). The SLR cannot be elicited by stimulating the brainstem or lumbar root (Ertekin et al., 1995; Suga et al., 2001). These properties indicate that the SLR is related to postural control, and that it could be a polysynaptic response of the agonist and antagonist organization system (Ertekin et al., 1995; Suga et al., 2001).

It is well known that the cerebellum plays an important role in the control of postural balance (Holmes, 1922). In our previous study (Suga et al., 2001), abnormal SLRs were detected in patients with cerebellar ataxia, including some patients with multiple system atrophy or other diseases in which extracerebellar systems were also involved. From these results, it is necessary to elucidate whether the cerebellofugal pathway or cerebellopetal pathway is responsible for the generation of the SLR.

Cerebellar signs are detected in nearly 100% of spinocerebellar ataxia type 6 (SCA 6) and late cortical cerebellar atrophy (LCCA) patients, whereas extracerebellar signs are mild and infrequent (Matsumura et al., 1997; Takahashi et al., 2004). However, central motor conduction time (CMCT) has been found to be prolonged in SCA6 patients (Chen et al., 2004; Lee et al., 2003), suggesting subclinical dysfunction of the pyramidal tract. Therefore, the purpose of this study was to determine the influence of the cerebellofugal pathway on the SPRs and SLRs elicited by TMS in patients with SCA 6 and LCCA.

2. Methods

2.1. Subjects

Nine patients (6 men and 3 women, ages 50–69 years) with cerebellar ataxia and 11 healthy subjects (5 men and 6 women, ages 50–64 years) were studied. Genetic analysis was performed on all patients: 5 were diagnosed as having SCA 6, while 4 had LCCA. All of the patients had mild to moderate limb and truncal ataxia (Table 1), but hyperreflexia, Babinski sign and extrapyramidal signs were not found. Three patients with localized cerebellar lesion were also tested: two of these had cerebellar arteriovenous malformation (AVM) and one had cerebellar infarcts (Table 2). These patients showed mild to moderate cerebellar ataxia but pyramidal or extrapyramidal signs were not present. Informed consent was obtained from each patient after the nature of genetic analysis and experiment had been fully explained. The experimental procedures were approved by the Ethics Committee of Graduate School of Medical Sciences, Kyushu University.

2.2. Magnetic cortical stimulation and electromyography (EMG) recording

The recording techniques have been described in detail (Suga et al., 2001). Briefly, a pair of surface EMG electrodes were placed about 3 cm apart over the long axis of the tibialis anterior (TA) and SOL muscles bilaterally. The EMG activity was bandpass filtered between 20 Hz and 3 kHz and amplified to 0.5–1.0 mV/div at a sampling rate of 1.7 kHz (Neuropack 8, Nihon Kohden).

A Magstim 200 magnetic stimulator (The Magstim Company) with a double-cone coil was placed with the center over the vertex, with the current flow in the brain being induced in an anterior–posterior direction. The strength of contraction of the TA or SOL muscles was controlled by monitoring prestimulus EMG activities by Neuropack 8, and more accurately with the real-time integrated EMG amplitude monitor using a personal computer (NEC). The stimulus intensity was changed in 5% steps of the maximum stimulator output. The MEPs from the TA and SOL were obtained at least four times for each condition. The two most stable responses were used for analysis. The onset latency was measured as the fastest latency of the four compound muscle action potentials (CMAPs) of each muscle, while the peak-to-peak amplitude was measured from the average of the two fastest responses (Suga et al., 2001).

Table 1
Demographic characteristics and the results of SLR in ataxic patients

Case No.	Diagnosis	Age	Sex	Cerebellar ataxia		SLR		
				Trunk	Lower limb (R/L)	Threshold (% output)	Lying	Standing
1	SCA 6	50	M	−3	−1/−2	45	N/N	N/N
2	SCA 6	54	F	−2	−1/−1	70	N/N	A/A
3	SCA 6	64	M	−2	−3/−2	>75	A/A	A/A
4	SCA 6	59	M	−2	−1/−1	>75	A/A	A/A
5	SCA 6	57	M	−1	−2/−2	50	N/N	A/A
6	LCCA	69	M	−1	−1/−1	>75	A/A	D/N
7	LCCA	51	F	−0.5	−0.5/−0.5	60	N/A	A/A
8	LCCA	54	M	−1	−2/−2	60	N/N	A/A
9	LCCA	63	F	−2	−3/−3	60	N/N	A/A

Ataxia: mild (−0.5 to −1), moderate (−2 to −3), severe (−4); R, right; L, left; N, normal; A, absent; D, delayed.