Table 1 Patient demographics

	Normal EEG	Abnormal EEG	*P*
Total number of patients	16	10	
Sex (n)[a]	F: 9, M: 7	F: 9, M: 1	NS
Mean age (years)[b]	43.3±12.9	25.9±9.4	<0.01
Illness duration before clozapine treatment (years)[b]	15.4±10.4	6.9±5.3	<0.01
Mean dose of clozapine (mg)[b]	378.1±179.8	305.0±131.7	NS
Length of clozapine treatment (months)[b]	13.3±10.4	17.5±11.4	NS

Notes: [a]Chi-square test; [b]Student's *t*-test. Data are presented as mean ± standard deviation.
Abbreviations: EEG, electroencephalography; F, female; M, male; NS, not significant.

Table 2 Clinical courses of patients with EEG abnormalities and seizure

Case	Age (years)	Sex	Time (weeks)	Dose (mg)	Seizure	EEG	Response
1	23	F	23	600	Myoclonus	Spike and slow wave, theta	None
			52	600	Few myoclonic	Spike, theta, delta	Start LTG 200 mg
			104	600	seizures	Slow wave	LTG 200 mg
			130	600	None	Theta	Increase to LTG 300 mg, decrease to clozapine 550 mg
					Tonic–clonic		
			>130	550		None	LTG 300 mg
					None		
2	19	F	18	350	Myoclonus	Spike and slow wave, theta	None
			24	500	None	Spike and slow wave, theta	None
			36	500	Few myoclonic		Start LTG 200 mg
			56	550	seizures	Spike and slow wave, theta	Decrease to LTG 150 mg
					None		
3	48	F	9	400	Myoclonus	Slow wave, spike and slow wave	None
			12	350	Myoclonus	Slow wave	Decrease to clozapine 300 mg
			26	300	None	Infrequent slow wave	None
4	24	F	8	300	Myoclonus	Spike and slow wave, delta	Start VPA 400 mg and increase to VPA 600 mg
			9	300	Myoclonus	Slow wave	VPA 600 mg
			12	300	Infrequent myoclonus	Slow wave	VPA 600 mg, decrease to clozapine 200 mg
					None		
			15	200	None	Infrequent slow wave	Decrease to VPA 400 mg
			23	200		None	VPA 400 mg
5	22	F	8	300	Tonic–clonic	Spike and slow wave, theta	Start VPA 400 mg and increase to VPA 600 mg
			>8	300	None	Theta	Increase to clozapine 400 mg
			18	400	Partial seizure	Theta	Increase to VPA 800 mg
			>18	400	None	None	VPA 800 mg
6	25	F	4	250	None	Theta	None
			8	350	Myoclonus	Slow wave, spike and slow wave	Decrease to clozapine 275 mg
			14	275	Myoclonus	Spike and slow wave, delta	Start VPA 600 mg
			15	275	Few and frequent myoclonic seizures	Spike and slow wave, delta	Increase to VPA 800 mg
			>15	275	None	None	VPA 800 mg
7	22	F	8	200	None	Spike and slow wave	None
			52	300	None	Few frequent theta	None
8	37	F	52	325	None	Spike and slow wave, theta	None
			104	325	None	Spike and slow wave, theta, delta	None
9	22	F	8	200	None	Spike and slow wave	None
			34	300	None	Infrequent spike and slow wave	None
10	17	F	8	125	None	Many theta waves	None
			52	300	None	Spike and slow wave	None

Abbreviations: EEG, electroencephalography; F, female; LTG, lamotrigine; VPA, valproate.

Case 1 experienced a myoclonic seizure 23 weeks after starting clozapine, and the dosage when the seizure occurred was 600 mg. Lamotrigine 200 mg was begun because myoclonus was still observed 52 weeks after clozapine initiation, although the incidence was low. Tonic–clonic seizure was observed 130 weeks after clozapine initiation. The lamotrigine dose was increased to 300 mg, and clozapine was decreased to 550 mg. After that, neither seizures nor EEG abnormalities were observed. Case 5 experienced tonic–clonic seizure 8 weeks after starting clozapine, when the dosage was 300 mg. Valproate 400 mg was initiated and subsequently increased to 600 mg. Although the seizures stopped, the patient's psychological symptoms exacerbated. Therefore, clozapine was increased to 400 mg. Partial seizure appeared 18 weeks after clozapine initiation despite treatment with valproate (600 mg). Increasing the valproate dosage to 800 mg effectively prevented further seizures without reduction of clozapine.

Comparison of PANSS (T) scores

PANSS (T) scores at baseline and at the last observation were compared between the normal and abnormal EEG groups. The mean baseline PANSS (T) scores were not significantly different between the two groups, but the mean score in the abnormal EEG group was significantly lower than that in the normal EEG group at the final follow-up (P=0.02). The response rate in the abnormal EEG group was higher than that in the normal EEG group, albeit not significantly (Table 3).

Discussion

Compared with other neuroleptics, clozapine appears to cause more seizures at therapeutic doses than the most epileptogenic of standard antipsychotic agents.[4] The mechanism of clozapine-induced seizure is not well understood. Typical antipsychotic drugs primarily target striatonigral dopamine D2 receptors, whereas clozapine blocks mesolimbic and cortical dopamine D4 receptors.[5] Mesolimbic structures are common sites of seizure onset. The selectivity of clozapine for mesolimbic dopamine receptors may explain its high epileptogenicity in comparison with other antipsychotic drugs.[4] Further possible mechanisms include its effects on other receptor types, including gamma-aminobutyric acid A, nicotinic acetylcholine, glutamate N-methyl-D-aspartate, serotonin 5-HT2A, and strychnine-sensitive glycine.[6–10] A dose-related risk of seizures with clozapine has been described in the literature, with reported risks of 0.6%–2% for doses <300 mg, 1.8%–4% for doses of 300–599 mg, and 5%–14% for patients taking 600–900 mg daily.[11–13] Previously identified risk factors for clozapine-induced seizures include rapid upward titration, preexisting seizure, and concurrent use of other epileptogenic medications.[14] Furthermore, clozapine-induced myoclonus can be a precursor to generalized seizure.[15,16] In the present study, six patients (23.1%) experienced myoclonic or tonic–clonic seizure. Only tonic–clonic seizure was experienced by two patients (7.7%). One patient with tonic–clonic seizure had previously had myoclonic seizure. The mean dose of clozapine was 380.3 mg (300–600 mg) at seizure onset. The incidence of seizure in this study was higher than in other reports in the literature.[11–13] These results may suggest that clozapine is more likely to cause seizures in the Japanese population. However, Kishi et al reported efficacy and tolerability of clozapine in Japanese patients with TRS and described the incidence of seizure in Japanese patients as 5.26%.[17] Our study involved a small number of patients, so we cannot conclude that Japanese patients with schizophrenia have a higher risk of seizure with clozapine use; additional studies with larger samples are needed to verify our observation. White and Van Cott[18] described the treatment of clozapine-associated seizures with dosage reduction and/or the addition of an antiepileptic drug. Praharaj et al[19] reported that it was prudent to add anticonvulsants immediately after the first seizure. Although valproate is recommended as the standard therapy, other drugs such as lamotrigine, gabapentin, and clonazepam are also useful.[15,16,19] Carbamazepine is contraindicated because of the increased risk of bone marrow suppression, and barbiturates may exacerbate sedation caused by clozapine.[1] Antiepileptic drugs that are highly protein-bound may displace clozapine from serum proteins.[4] However, why antiepileptic drugs are effective for clozapine-induced seizure without exacerbating psychological symptoms is unclear. All patients who experienced seizures in this study were successfully treated either with antiepileptic drugs or no drug with a small or no reduction of clozapine. Therefore, we believe that there is no need to discontinue clozapine in patients who experience seizures.

Table 3 Comparison of clinical results between the normal and abnormal EEG groups

	Normal EEG	Abnormal EEG	P
Baseline PANSS (T)	99.4±27.8	87.9±15.2	NS
Last-observation PANSS (T)	59.1±12.1	46.7±11.3	0.02
Response rate (%)	37.4±16.9	46.6±9.9	NS

Note: Data are presented as mean ± standard deviation.
Abbreviations: EEG, electroencephalography; NS, not significant; PANSS (T), total Positive and Negative Syndrome Scale score.

Studies have reported that EEG abnormalities associated with clozapine treatment range from 16%–74%.[2,20] Among typical and atypical antipsychotics, clozapine was most strongly associated with EEG abnormalities (clozapine: 47.1%, olanzapine: 38.5%, risperidone: 28.0%, typical neuroleptics: 14.5%, quetiapine: 0.0%).[21] We found that the incidence of clozapine-induced EEG abnormalities in Japanese subjects with schizophrenia was 38.5%. It has been reported that clozapine-induced EEG abnormalities occur in a dose-dependent manner and correlate with the serum level of clozapine.[2,22–24] However, Centorrino et al[21] did not find a relationship between dose and EEG abnormalities. Goyal et al[25] reported EEG abnormalities in 61.9% of patients receiving clozapine at a dose of 100 mg or less. In this study, the mean dose of clozapine at the occurrence of EEG abnormalities varied from 125–600 mg (mean 305 mg/day) and was lower than that in the normal EEG group (mean 378.1 mg/day), albeit not significantly. In one-half of the patients with EEG abnormalities, the clozapine dose was <300 mg. Thus, the relationship between clozapine dosage and EEG abnormalities remains controversial. Haring et al[24] described EEG abnormalities in 52% of patients and reported that these were dependent on plasma levels; they also determined that dose was not statistically related to EEG abnormalities. It is necessary to identify the relationship of EEG abnormalities and clozapine serum level. Centorrino et al[21] determined that significant risk factors for EEG abnormalities were the use of various antipsychotic drugs, including clozapine; age over 40 years; and hypertension. On the other hand, Chung et al[26] suggested that younger patients on the same clozapine dosage as older patients were more likely to have EEG abnormalities. In this study, patients in the abnormal EEG group were significantly younger than those in the normal EEG group. The abnormal EEG group also had a significantly shorter illness duration before clozapine treatment than the normal EEG group. These results indicate that EEG abnormalities may appear in patients who started clozapine treatment in the early phase of schizophrenia or when they were young.

Risby et al[3] reported that EEG abnormalities that developed after clozapine treatment appeared to be associated with good clinical response. In this study, there was no significant difference in baseline PANSS (T) scores between the normal and abnormal EEG groups. However, the mean PANSS (T) score from the last observation was significantly lower in the abnormal EEG group, and the response rate of the abnormal EEG group was also higher, albeit not significantly, compared to that of the normal EEG group. These results indicate that EEG abnormalities that appear after clozapine treatment are associated with a good clinical response. Another study reported that pretreatment intrahemispheric asymmetry on EEG predicted the short-term response to clozapine in patients with schizophrenia.[27] The change in theta frequency in quantitative EEG, and particularly changes in the midline electrodes over the frontocentral scalp area, might be a more sensitive indicator for evaluating clozapine treatment adequacy.[28] Welch et al[2] reported that the appearance of paroxysmal discharges with bursts of slow waves, polyspike bursts, and spike and sharp waves indicated a high risk for convulsions. Thus, the presence of EEG abnormalities is not necessarily indicative of a good response to clozapine. However, EEG results may be useful for adjusting clozapine dose if it is clarified which EEG results are related to good clinical responses or convulsions.

Conclusion

EEG abnormalities may appear in younger patients, and our findings indicate that there is no need to discontinue clozapine when seizures occur. EEG abnormalities that appeared after clozapine treatment were associated with a good clinical response.

Acknowledgments

We are grateful to all the participants in this study. We also thank Kazumi Shimizu for providing the symptom assessment.

Disclosure

This work was supported by Health and Labor Sciences Research Grant Number 3000000301. The authors report no other conflicts of interest in this work.

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http://dx.doi.org/10.5665/sleep.3334

Association between Heart Rate Variability, Blood Pressure and Autonomic Activity in Cyclic Alternating Pattern during Sleep

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Study Objectives: Cyclic alternating pattern (CAP) is frequently followed by changes in heart rate (HR) and blood pressure (BP), but the sequential associations between CAP and autonomic nerve activity have not been studied. The study aimed to reveal the precise changes in heart rate variability (HRV) during phase A of the CAP cycle.

Design: Polysomnography was recorded according to the CAP Atlas (Terzano, 2002), and BP and electrocardiogram were simultaneously recorded. The complex demodulation method was used for analysis of HRV and evaluation of autonomic nerve activity.

Setting: Academic sleep laboratory.

Participants: Ten healthy males.

Measurements and Results: The increase in HR (median [first quartile – third quartile]) for each subtype was as follows: A1, 0.64 (-0.30 to 1.69), A2, 1.44 (0.02 to 3.79), and A3, 6.24 (2.53 to 10.76) bpm (A1 vs. A2 $P < 0.001$, A1 vs. A3 $P < 0.001$, A2 vs. A3 $P < 0.001$). The increase in BP for each subtype was as follows: A1, 1.23 (-2.04 to 5.75), A2, 1.76 (-1.46 to 9.32), and A3, 12.51 (4.75 to 19.94) mm Hg (A1 vs. A2 $P = 0.249$, A1 vs. A3 $P < 0.001$, A2 vs. A3 $P < 0.001$). In all of phase A, the peak values for HR and BP appeared at 4.2 (3.5 to 5.4) and 8.4 (7.0 to 10.3) seconds, respectively, after the onset of phase A. The area under the curve for low-frequency and high-frequency amplitude significantly increased after the onset of CAP phase A ($P < 0.001$) and was higher in the order of subtype A3, A2, and A1 ($P < 0.001$).

Conclusions: All phase A subtypes were accompanied with increased heart rate variability, and the largest heart rate variability was seen in subtype A3, while a tendency for less heart rate variability was seen in subtype A1.

Keywords: Cyclic alternating pattern, heart rate variability, blood pressure, complex demodulation method, autonomic nerve activation

Citation: Kondo H; Ozone M; Ohki N; Sagawa Y; Yamamichi K; Fukuju M; Yoshida T; Nishi C; Kawasaki; Mori; Kanbayashi T; Izumi M; Hishikawa Y; Nishino S; Shimizu T. Association between heart rate variability, blood pressure and autonomic activity in cyclic alternating pattern during sleep. *SLEEP* 2014;37(1):187-194.

INTRODUCTION

Arousal reactions are important for clarifying the physiological and pathological mechanisms of natural sleep and sleep disorders. After a report published in 1992 by the ASDA (American Sleep Disorders Association) on EEG arousals, EEG arousals have been used as a marker of sleep fragmentation.[1] According to the ASDA report, an EEG arousal is an abrupt shift in EEG frequency, which may include theta, alpha, and/or EEG frequencies greater than 16 Hz. Asynchronous waves maintained for 3 seconds were defined as arousals, but high-amplitude slow waves, such as K complexes (KC) and delta bursts, were not counted as arousals. On the other hand, high-amplitude slow waves are often observed before the appearance of the asynchronous low-voltage mixed waves in NREM sleep. These periodic EEG complexes were defined as cyclic alternating patterns (CAP) by Terzano in the 1980s.[2]

Submitted for publication October, 2012
Submitted in final revised form October, 2013
Accepted for publication October, 2013

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CAP is considered a marker of sleep instability and has been used for the evaluation of sleep in various sleep disorders and sleep changes with hypnotics.[3-16] A CAP cycle, which is the minimum unit of CAP, consists of two phases: phase A and B. During phase A, high voltage waves appear and diminish synchronously. The period following phase A, in which low amplitude EEG is present, is defined as phase B. Phase A is scored within a CAP sequence only if it preceded and/or follows another phase A within the 2-60 seconds temporal range. In addition, phase A is divided into three subtypes (A1, A2, A3) on the basis of the ratio of the synchronous high-amplitude slow wave period to the whole duration of phase A (A1: > 80%, A2: 50% to 80%, A3: < 50%).[2,17] Subtype A1 is frequently observed in the first part of the sleep cycle, in slow wave sleep, and subtypes A2 and A3 appear before the onset of REM sleep in healthy volunteers.

The components of arousal reactions include not only the changes in EEG frequency but also autonomic nerve activity involved with HR, BP, and skeletal muscle tension.[18] In NREM sleep, external stimuli induce not only high-amplitude slow wave components in EEG (e.g., K complexes [KC]), but also autonomic nerve reactions, such as the increase of HR and BP.[19,20] These autonomic nerve activities are also observed in the occurrences of KC and delta bursts with no external stimuli.[19,21-23] EEG shifts followed by autonomic nerve activity also appear before or simultaneously with leg movements in periodic leg movements (PLM).[3,24-27] Thus, EEG shifts,

Table 1—Demographic data (n = 10)

	Median	IQR
Age, y	21.0	4.0
Height, cm	175.0	7.4
Weight, kg	66.4	10.8
BMI, kg/m^2	21.5	2.5
PSQI GS	3.5	2.0
ESS	4.5	4.0

IQR, interquartile range; BMI, body mass index; PSQI GS, Pittsburgh Sleep Quality Index Global Score; ESS, Epworth Sleepiness Scale

including both synchronous high-amplitude slow wave and asynchronous components, are considered to have significant associations with the autonomic nerve and skeletal muscle activities in physiological and pathological conditions.

Heart rate variability (HRV) is often used for evaluating autonomic nerve activity.[28] There are a number of reports that have previously analyzed HRV in CAP using frequency analysis.[29,30] According to these reports, the balance of autonomic nerve activity is important, and the results indicate sympathetic nerve dominance during CAP sequences versus non-CAP sequences, even for the same sleep stages. However, detailed analysis of the autonomic nerve activity involved with the increased heart rate in KC and delta bursts has not been reported. Furthermore, the duration of phase A including KC and delta bursts typically lasts for 2 to 10 seconds. However, the frequency analysis logically needs data from at least 40 seconds.[28] Thus, it is impossible to assess the short, rapid autonomic nervous reactions by the standard frequency analysis. Therefore, we continuously measured both HR and BP using nocturnal polysomnography, and applied the complex demodulation method (CDM) for sequential analysis of HRV.[31,32] The CDM makes it possible to analyze the time course of amplitude in a specific frequency band and to evaluate the autonomic nerve activity with high time resolution.

This study aims to reveal the relationship between CAP and the time course of HRV, and study the physiological significance of CAP.

METHODS

Subjects

We evaluated 10 healthy males with a median age (IQR; interquartile range) of 21.0 (4.0) years. Exclusion criteria included the following: habitual drinker, habitual smoker, having physical or psychiatric diseases. Habitual sleep state was evaluated by the Pittsburgh Sleep Quality Index (PSQI).[33,34] For the PSQI, the median (IQR) global score was 3.5 (2) points, which matched the average global score of the general Japanese population.[35] Subjective daytime sleepiness was assessed by the Epworth Sleepiness Scale (ESS).[36] The median (IQR) of ESS was 4.5 (4.0) points, which was equivalent to that of healthy Japanese volunteers (Table 1).

All subjects gave written informed consent, which was conducted with the approval of the Ethics Committee of Akita University School of Medicine.

Polysomnography

Polysomnography was conducted for 8 h, in accordance with the habitual sleep time of each subject. To determine the sleep stages and the CAP parameters, both unipolar induction electrodes (C3-A2, C4-A1, O1-A2, O2-A1) and bipolar induction electrodes (Fp1-F3, F3-C3, C3-P3, P3-O1, Fp2-F4, F4-C4, C4-P4, and P4-O2) were attached to each subject. Electrodes were also attached to obtain electromyograms of the chin and anterior tibialis muscles, electroculograms, and ECGs. To measure the flow of air, airflow sensors were attached to the nose and mouth using the thermocouple method. To record breathing movements, respiratory effort sensors were attached to the chest and abdomen using the piezoelectric method. Body position sensors, snore sensors, and pulse oximeters were attached to record body position, snoring, and arterial oxygen saturation, respectively, of each subject.

The Neurofax EEG-1524 (Nihon Kohden Corporation, Tokyo, Japan) was used to record digital electroencephalographs. The data obtained were then imported and recorded on a computer using the BioSignal Acquisition System (NoruPro Light Systems, Tokyo, Japan). The sampling rates for recording were as follows: EEG, 1000 Hz; electromyograms, 200 Hz; snore sensors, 200 Hz; ECG, 1000 Hz; breathing movements, body position sensors, pulse oximeters, and pressure sensors, 100 Hz.

NightOwl Professional (NoruPro Light Systems) was used for throughout the analysis of sleep stages; 1 epoch was defined as 30 seconds. All evaluations were based on the criteria by Rechtschaffen and Kales.[37] EEG arousals and periodic limb movements were scored according to the AASM Scoring Manual.[38]

Blood Pressure Measurement

Portapres Model-2 (Finapres Medical Systems BV, Amsterdam, Netherlands) was used for consecutive blood pressure measuring, using plethysmography. Cuffs were fixed on the first and second fingers of the left hand, and BP measurement was alternated between the 2 fingers every hour. Pressure wave data obtained were imported by analog output with a sampling rate of 100 Hz and were recorded on an electroencephalograph by digital input.

CAP, Heart Rate, and Blood Pressure Analysis

For the evaluation of CAP, PSG data were visually scored by T.Y., A.K., and H.K., based on the scoring rules written by Terzano,[2] with the aid of CAP analysis software (NoruPro Light Systems). CAP analysis was also used for analyzing variations in heart rate and systolic pressure for each CAP subtype.

RR intervals were calculated in accordance with the peaks of R waves in lead II of the electrocardiogram. RR intervals < 300 ms or > 1700 ms were excluded in order to eliminate the influence of artifacts. In regards to BP, systolic blood pressure was detected by the peaks of pulse waves based on intermittent automatic calibration waves of a sphygmomanometer for 3 s with 90 s intervals. The BP data obtained during calibration were excluded for analysis.

The variations in heart rate and blood pressure were analyzed from 15 s before to 60 s after the onset of phase A. The moving averages were calculated using datum points with 0.1 second intervals; average values between 1.5 s before to

Table 2—Polysomnography findings

	Median	IQR
Total recording time, min	481.3	11.9
Total sleep time, min	456.8	37.9
Sleep efficiency, %	94.9	6.9
WASO , min	9.8	18.4
Sleep latency, min	4.5	10.3
Time in each stage, min		
REM	71.3	38.5
Stage 1	60.3	28.0
Stage 2	241.0	29.4
Slow wave sleep	56.3	34.1
Movements	6.3	2.9
Percent of TST, %		
REM	16.1	7.0
Stage 1	13.1	6.4
Stage 2	54.0	2.6
Slow wave sleep	12.2	8.2
Movements	1.4	0.6
REM latency, min	73.8	24.3
AHI , n/h	0.1	0.3
Arousal index, n/h	15.7	8.0
PLMS index, n/h	1.2	4.0

IQR, interquartile range; WASO, wake after sleep onset; TST, total sleep time; Movements, major body movements; AHI, apnea-hypopnea index; PLMS, periodic limb movements of sleep.

Table 3—CAP parameters

	Median	IQR
CAP Rate, %	36.4	21.6
CAP Time, min	140.4	82.1
CAP Cycle, n	334.0	212.3
A1, n	218.5	92.5
Ratio, %	73.2	23.3
CAP index, n/h	35.8	14.7
A2, n	62.0	77.8
Ratio, %	18.2	11.2
CAP index, n/h	10.0	12.8
A3, n	36.0	38.8
Ratio, %	11.0	12.2
CAP index, n/h	5.7	6.2

IQR, interquartile range; CAP, cyclic alternating pattern; CAP Rate was calculated as the ratio of total CAP sequence time to whole non-REM sleep time; CAP Time was calculated as total CAP sequence time; CAP Cycle indicates total CAP cycle counts; CAP Ratio represents the percentage of the number of CAP cycle counts for each subtype; CAP index represents the number of CAP cycle counts for each subtype per hour of NREM.

1.5 s after the datum points were calculated, and the amount of change was analyzed on the basis of the average 5 s prior to the onset of phase A.

HRV Analysis

We applied the CDM[31,32] for sequential analysis of autonomic nerve activity. Hayano proposed and established the use CDM for assessment of frequency shifts in HR and BP variability.[32] CDM is suited for continuous assessment of time-dependent changes in amplitude in the rhythmic components of predefined frequency bands. The RR intervals of the ECG were analyzed from 15 s before to 60 s after the onset of phase A using the CDM. The amplitude values for the low-frequency content (LF: 0.04 to 0.15 Hz) and the high-frequency content (HF: 0.15 to 0.4 Hz) were calculated continuously.

The amplitude value for the HF content is considered an indicator of parasympathetic nerve activity, while the amplitude value for the LF content reflects both sympathetic and parasympathetic nerve activity.[28] The area under the curve (AUC) of the LF and HF amplitude values was calculated for the first 20 s of CAP. In order to compare the changes before and after the CAP, the AUC for the 5 seconds leading to the CAP onset were quadrupled and compared with that of values for the 20 s after the CAP onset.

Statistics

PASW Statistics version 17.02 for Windows (SPSS Japan Inc., Tokyo, Japan) was used for statistical processing. Most data sets in this study did not indicate normal distribution. The data of HRV in subtype A3 indicated logarithmic normal distribution, but the others did not. Thus, data were shown as median (IQR) or median (first quartile – third quartile), and nonparametric statistics were applied: Kruskal-Wallis H statistic was used for comparisons among the 3 groups in CAP subtypes. Scheffe test was used for multiple comparisons. Wilcoxson signed rank test was used for comparing the AUC of the LF and HF amplitude values before and after the CAP onset. The level of significance was set at 0.05 for each test.

RESULTS

According to the sleep parameters, the sleep structures of our subjects were considered normal, and sleep related respiratory disorders and/or periodic limb movement disorders were not found (Table 2).

CAP parameters (Table 3) had large individual variations; the median CAP rate (IQR) was 36.4% (21.6%) and the median CAP cycle counts was 334.0 (212.3). Some subjects have higher CAP rates than those reported in healthy subjects from a similar age group.[39] Three of the 10 subjects had a CAP > 50%, and in 2 of the 3 subjects, the percentage of the number of subtype A2 and A3 was > 50%. The higher CAP values in this study might be due to influences on the sleep quality by the attachments of cuffs on fingers and a monitor device on the arm.

The number of phase As totaled 3527 in 10 subjects. R waves of ECG were well detected, and HRV could be calculated in 3262 of the phase As. Changes in BP could be evaluated without the influence of finger change and/or intermittent automatic calibration in 2474 of the phase As.

HR increased immediately after the beginning of CAP. The increase in HR for each subtype was as follows: A1, 0.64 (-0.30 to 1.69); A2, 1.44 (0.02 to 3.79); and A3, 6.24 (2.53 to 10.76) bpm ($H = 516.9$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P < 0.001$,

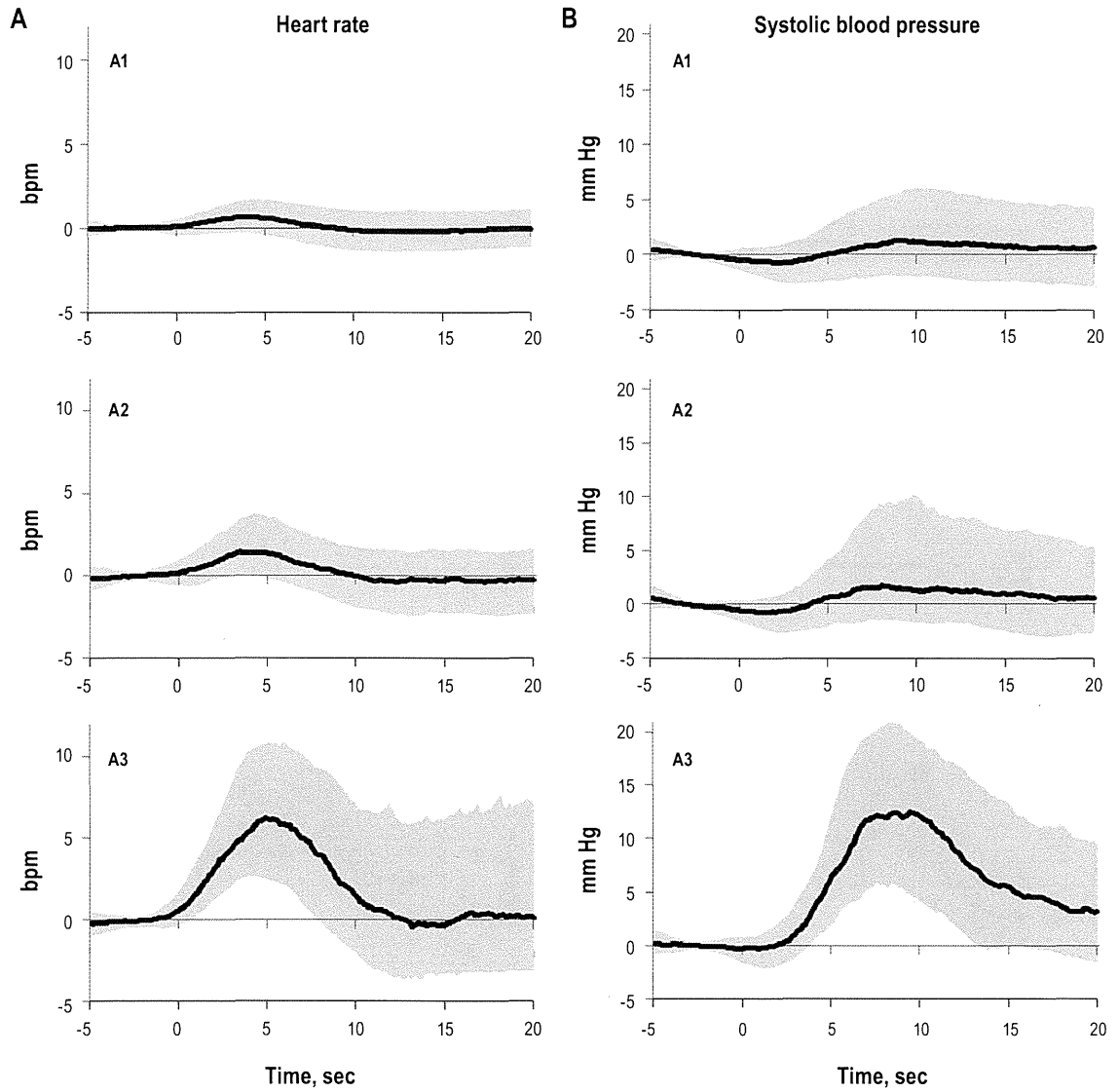

Figure 1—The time course of heart rate and systolic blood pressure changes before and after the onset of CAP. The median value (black line) of each CAP subtype was calculated **(A, B)** and graphed. Gray shadows indicate interquartile range of heart rate and systolic blood pressure. Zero seconds indicates the onset of CAP. The peak time of systolic blood pressure is delayed by approximately 4 seconds compared with that of heart rate. The increase in both heart rate and systolic pressure is higher in the order of subtype A3, A2, and A1.

A1 *vs.* A3 P < 0.001, A2 *vs.* A3 P < 0.001). In all of phase A, the peak values for HR appeared at 4.2 (3.5 to 5.4) s after the onset of phase A (Figure 1A).

BP transiently decreased after the onset of phase A, and then gradually increased. The decrease in BP for each subtype was as follows: A1, -1.40 (-3.10 to -0.07); A2, -1.44 (-3.15 to -0.24); A3, -0.89 (-2.82 to 0.46) mm Hg (H = 11.1, df = 2, P = 0.004, A1 *vs.* A2 P = 0.719, A1 *vs.* A3 P = 0.294, A2 *vs.* A3 P = 0.162). The nadir values for BP appeared at 1.5 (0.0 to 2.8) sec after the onset of phase A. The increase in BP for each subtype was as follows: A1, 1.23 (-2.04 to 5.75); A2, 1.76 (-1.46 to 9.32); and A3, 12.51 (4.75 to 19.94) mm Hg (H = 201.7, df = 2, P < 0.001, A1 *vs.* A2 P = 0.249, A1 *vs.* A3 P < 0.001, A2 *vs.* A3 P < 0.001). The time courses in all subtypes of CAP were similarly observed. Concerning the variations in HR and BP, the magnitude of subtype A3 was the largest, and the magnitude of subtype A1 was the smallest among the 3 CAP subtypes. In all subtypes of phase A, the peak values for BP appeared at 8.4 (7.0 to 10.3) s after the onset of phase A (Figure 1B).

As a result of evaluation of autonomic nerve activity using the CDM, we observed that the amplitude of LF had 2 peaks within 10 s after the onset of phase A. The AUC of LF for the 20 s after the onset of phase A was significantly higher than before the onset of phase A in all CAP subtypes. As for the AUC for LF amplitude before *vs* after the onset of phase A for each subtype, A1 was 491.3 (318.4 to 759.4) *vs.* 559.0 (387.9 to

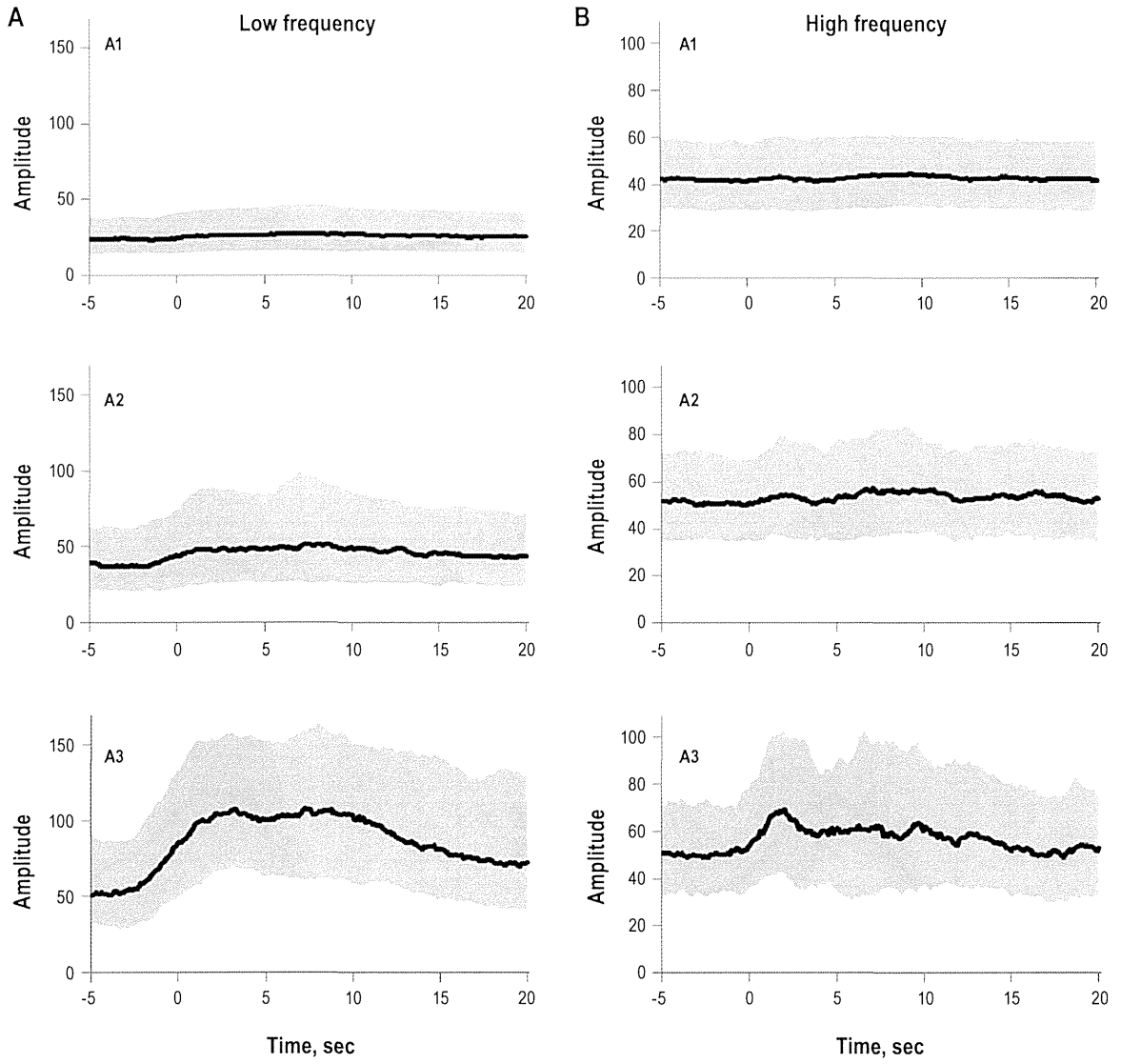

Figure 2—Comparison of time course in heart rate variability before and after the onset of CAP phase A. The median value (black line) of each CAP subtype was calculated **(A, B)** and graphed. Gray shadows indicate interquartile range of low frequency (LF: 0.04 to 0.15 Hz) and high frequency (HF: 0.15 to 0.4 Hz). Zero seconds indicates the onset of phase A. The amplitude of LF was the highest in subtype A3, followed by subtypes A2 and A1.

878.1) ($P < 0.001$); A2 was 787.1 (502.1 to 1299.1) *vs.* 1044.0 (644.3 to 1688.2) ($P < 0.001$); and A3 was 1226.9 (772.5 to 1903.9) *vs.* 2087.1 (1373.1 to 2744.0) ($P < 0.001$). The AUC for LF amplitude before the onset of phase A had significant differences among the 3 subtypes ($H = 548.3$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P < 0.001$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P < 0.001$); it was the highest in A3 and the lowest in A1. Similarly, the AUC for LF amplitude after the onset of phase A had significant differences among the 3 subtypes; it was higher in the order of A3, A2, and A1 ($H = 895.7$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P < 0.001$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P < 0.001$; Figure 2A).

The HF amplitude values changed more smoothly than those for LF. The HF amplitude values in every CAP subtype were significantly higher than those before the onset of phase A for the first 20 s after the onset of phase A. The AUC for HF amplitude before *vs* after the onset of phase A for each subtype was as follows: A1, 872.6 (631.2 to 1146.7) *vs.* 924.0 (679.4 to 1175.3) ($P < 0.001$); A2, 1054.7 (779.6 to 1401.2) *vs.* 1186.1 (910.2 to 1466.3) ($P < 0.001$); A3, 1037.5 (778.6 to 1443.5) *vs.* 1367.0 (1032.1 to 1672.4) ($P < 0.001$). The AUC for HF amplitude value had significant differences before the onset of phase A among the 3 CAP subtypes ($H = 136.1$, $df = 2$, $P < 0.001$), but the value of A3 was not significantly different to that of A2 (A1 *vs.* A2 $P < 0.001$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P = 0.999$). Concerning the AUC for HF amplitude after the onset of phase A, it was higher in the order of A3, A2, and A1 ($H = 398.9$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P < 0.001$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P < 0.001$) (Figure 2B).

DISCUSSION

Time Course of Heart Rate, Blood Pressure, And Heart Rate Variability after the Onset of CAP

To our knowledge, this is the first manuscript that analyzes the time course of HRV for each CAP subtype using a high time resolution CDM. In healthy male subjects, the HR started to increase at the onset of CAP. BP transiently decreased after the onset of phase A, and then gradually increased. The result of HRV analysis using the CDM indicates that the amplitude of LF was larger than that of HF, and its time course showed two peaks, with the latter peak corresponding to the peak time of blood pressure. Although time courses of increases in HR, BP, and HRV are similar among the three CAP subtypes, the magnitudes of the variations were larger in the order of subtype A3, A2, and A1, demonstrating that the degree of HR, BP, and HRV varied among subtypes of CAP, and that A3 induced the most prominent effects followed by A2. We thus attempted to interpret and discuss the data in detail.

Why Does Blood Pressure Increase Later than Heart Rate after the Onset of CAP?

Concerning the responsiveness of HR and BP (i.e., the time until HR and BP peaked), our findings coincided with previous reports of K complex (KC), HR, and BP changes. HR began to increase at the appearance of KC, and reached the peak value on the third beat, whereas BP increase was relatively delayed and reached the peak value on the sixth beat.[19,21]

The reaction time is determined by the network conduction velocity of the autonomic nervous system and the responsiveness of the end effectors. The reaction time is considered the same between individuals. It can explain why the BP increased later than the peak HR, based on the differences between the reaction times of the end effectors. The parasympathetic nerve activity has a rapid reaction time system (approximately 10^{-3} seconds) by the ion-channel type reaction. On the other hand, the cardiac sympathetic nerve activity has a slower reaction time system (from 10^{-1} to 10^{0} seconds) and is characterized by long reaction time duration, which is due to a series of reactions induced by the intracellular second messengers occurring through G protein-coupled receptors. The rise of blood pressure is affected by the increase of HR, the heightened vascular resistance due to the arteriole shrinking, and the cardiac contractile force. Thus, it may take approximately 8 seconds to reach the peak blood pressure as a consequence all of these vital reactions.

Muscle sympathetic nerve activity (MSNA) is a sympathetic impulse activity, which induces vascular shrinking by controlling the vascular smooth muscle in skeletal muscle and also contributes to the regulation of blood pressure. Previous studies using microneurography reported that MSNA started to rise at the second beat (approximately 1.2 s after the appearance of KC).[20] The time lapse of MSNA from KC may partly explaine the decrease of BP after the onset of phase A.

Comparison of this Study with the Time Course of Autonomic Nerve Activity Induced by PLM

Although our study reports the time course of HRV for each CAP subtype for the first time, sequential measuring of HRV to clarify of the autonomic nerve activity in PLM has been previously reported.[27] In a report by Guggisberg, the time course of LF showed two peaks at 2 and 6 seconds after the onset of PLM, and the power of LF was higher in the latter peak.[27] Interestingly, the delta power of EEG started to increase approximately 2 seconds before the onset of PLM. Sforza also reported a similar increase of delta power of EEG.[26] Moreover, reports showed CAP subtypes A2 and A3 were frequently observed in patients with PLM, and the delta power of EEG appeared prior to or in concurrence with the emergence of PLM.[11,25] Considering the time difference (2 s) between the emergences of PLM and the slow wave, the estimated time peak of LF will be 4 and 8 seconds after the occurrence of slow wave; these values coincided with our findings.

It was not clear why the time course of LF had two peaks and reached the maximum level at 8 seconds after the onset of CAP. Previous studies reported that MSNA showed a transient activation at 1.2 seconds after KC appearance.[20] This transient activation is considered the first peak of LF after the onset of CAP in our study. It was reported that MSNA was activated transiently after the onset of KC, then returned to baseline, and was suppressed at the sixth beat where the BP reached a peak.[21] However, our data and those of Guggisberg showed that the power/amplitude values for LF represented the latter peaks in these periods where MSNA were suppressed. Therefore, it is uncertain whether the latter peak of LF really reflected the sympathetic nerve activity. Baroreceptor reflex leads to the increase of the cardiac parasympathetic nerve activity. In this period, the power/amplitude values for HF actually rose. As the parasympathetic nerve activity is also reflected in the power of LF, the increase of LF power after the onset of CAP, especially in the latter peak, may represent the parasympathetic nerve activity rather than the sympathetic nerve activity.

On the other hand, the amplitude of HF increased approximately 8 seconds after the onset of phase A. Guggisberg reported that the power of HF slightly increased for a little while after the emergence of PLM, and peaked at about 6 seconds after the emergence of PLM (approximately 8 s after the occurrence of delta power of EEG).[27] It is believed that these results reflect the increase of the cardiac parasympathetic nerve activity induced by the baroreceptor reflex.

In our study, the amplitude increase of HF was observed approximately 3 seconds after the onset of phase A. The same amplitude of HF was not observed in Guggisberg's report. This may be due to the difference in the CAP occurrences; we analyzed spontaneous CAP, while Guggisberg specifically analyzed CAP induced by PLM. Further studies will be needed to clarify the difference between CAP induced spontaneously and secondarily.

Study Limitations

Although the CDM has a higher time resolution than frequency domain analysis, the amplitude values for LF are influenced by the ± 8 seconds of the evaluation point; those for HF are also influenced by the ± 3 seconds around the point. Thus, the transient changes in LF before the onset of phase A could be affected by the subsequent changes.

The power/amplitude value for HF indicates parasympathetic nerve activity, and the power/amplitude value for LF reflects both sympathetic nerve activity and parasympathetic nerve

activity. There are some studies evaluating the predominant state of sympathetic nerve using the ratio of HF to LF power/amplitude value. But these procedures are often controversial, because the time range required for measuring is different between the amplitude values for LF and HF. Thus, we should note that HRV analysis is an indirect assessment method of autonomic nerve activity.

Moreover, it should be noted that parasympathetic nerve activity is reflected not only in the HF region. Parasympathetic nerve activity reflects respiratory sinus arrhythmia (RSA). If respiratory frequency is more than 9/min, RSA is recognized in the HF region. If respiratory frequency decreases below 9/min, RSA is recognized in the LF region. The minimum of respiratory frequency was 10.8/min in this study. Thus, we believe that we could successfully assess the RSA reflected in the HF region.

As for statistical analysis, the values of measurements almost represented nonparametric distributions despite logarithmic transformation. Thus, we could not employ a suitable analysis method taking sleep stages, sleep cycles, and factors between individuals into consideration, because of use of nonparametric analysis. In this study CAP parameters had large individual differences. Therefore, more subjects with enough CAP events are needed to assess HRV that takes the influences of sleep stages and sleep cycles into consideration.

Relationship between the Autonomic Network and CAP

In regard to the amplitude before the onset of phase A, HF was similar among the three CAP subtypes. In terms of LF, subtype A3 was the largest, and subtype A1 was the smallest. In the study using the low resolution brain electromagnetic tomography (LORETA), Ferri revealed the distinct difference in the areas of the cortical generators between subtype A1 and A3; subtype A1, anterior frontal regions; A3, the parietal-occipital areas.[40] It was also reported that the amount of CAP subtype A2 and A3 was highly correlated to the arousal index.[39] However, that of subtype A1 was not. Subtype A1 instead correlated positively with the percentages of slow wave sleep, in which a tendency of parasympathetic nerve activity dominancy was frequently observed.

Our results suggest the functional interaction between the central autonomic network and the thalamo-cortical network,[41] which is related to the occurrence of high-amplitude slow waves. The central autonomic network[42] includes the limbic system and the area from the hypothalamus to the medulla oblongata and the midbrain, which regulates autonomic nerve activity. Future studies that anatomically clarify the connections between the central autonomic network and the thalamo-cortical system are needed, as well as studies that more directly evaluate the autonomic nerve activity in the occurrence of CAP, such as by MSNA measuring.

In conclusion, this is the first report that describes the sequential time course of HRV around the occurrence of CAP. We simultaneously observed rapid and transient HRV and CAP, and the largest HRV was seen in subtype A3. Since the sleep-wake controlling system has a high association with the regulation of autonomic nerve activity and is responsible for the maintenance of this behavioral state, further clarification of functional significances of the findings is warranted to understand the physiological significance of sleep and the pathological mechanisms of sleep related disorders.

ACKNOWLEDGMENTS

The authors thank Dr. Sachiko Uemura for her statistical review, Ms. Mari Matsumoto for her English editing, and Ms. Namiko Matsubuchi, Dr. Takaubu Takemura, and Dr. Fumi Takemura for their help with data collection.

DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

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Psychiatry and Clinical Neurosciences 2014; 68: 188–196 doi:10.1111/pcn.12115

Regular Article

Psychosocial functioning is correlated with activation in the anterior cingulate cortex and left lateral prefrontal cortex during a verbal fluency task in euthymic bipolar disorder: A preliminary fMRI study

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Aim: Cognitive impairment may account for functional and occupational disability in patients with bipolar disorder even during periods of euthymia. While imaging suggests structural, neurochemical, and functional abnormalities in bipolar disorder patients, the pathophysiology of these deficits has not been elucidated. It was hypothesized that euthymic bipolar patients would have different cortical activation during a verbal fluency task compared to healthy controls, and that psychosocial functioning would be associated with prefrontal cortical activation during the task in the bipolar group.

Methods: Ten euthymic bipolar patients and 10 healthy control participants (matched for age, gender, and years of education) underwent functional magnetic resonance imaging (fMRI) during a verbal fluency task, tapping task and visual task. Correlational analysis between the fMRI brain activation and clinical variables of the participants, including Global Assessment of Functioning (GAF) score, was performed.

Results: Compared to the controls, euthymic bipolar patients had significantly greater activation in the bilateral precuneus with similar behavioral performance during the verbal fluency task. There were no significant differences between the groups for the visual task or the simple motor task. Activation in both the left anterior cingulate cortex (ACC) and the left dorsolateral prefrontal cortex (PFC) were significantly positively correlated with GAF score in the euthymic bipolar patients.

Conclusion: Both the ACC and lateral PFC regions are components of a neural network that plays a critical role in psychosocial functioning, and are often found to be affected in bipolar patients.

Key words: bipolar disorder, euthymia, functional magnetic resonance imaging, prefrontal cortex, psychosocial functioning.

ALTHOUGH PATIENTS WITH bipolar disorder (BD) have historically been characterized as returning to baseline function between affective episodes,[1] it is increasingly apparent that this view is somewhat inaccurate. Euthymic BD patients, although clinically in remission, often continue to be functionally compromised.[2] Some studies point to a significant degree of psychosocial dysfunction even when patients are euthymic.[3,4] Other reports have emphasized the impact of cognitive dysfunction on psychosocial functioning in BD patients.[5,6] In addition, psychosocial functioning seems to be more strongly associated with cognitive impairment than

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Received 27 September 2012; revised 15 July 2013; accepted 29 August 2013.

most other clinical variables.[7] Verbal fluency is an aspect of higher executive function, a spectrum of cognitive processes that are essential to control and regulate lower-level processing and goal-directed behavior.[8] A recent meta-analysis of cognitive deficits in euthymic BD patients found a small impairment in phonetic fluency.[9]

Neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), are now widely used to study BD.[10] Studies of executive functioning in BD patients have included working memory tasks, continuous performance tasks, Stroop and language tasks. Some functional neuroimaging studies found blunted activation of the prefrontal cortex (PFC) during cognitive tasks in BD patients.[11,12] Other studies did not find hypoactivation but reported enhanced or unchanged activation.[13,14] Although there has been variability of results depending on the clinical state of the patients and the task design, the PFC has been consistently involved.[15] Psychosocial functioning is a sophisticated construct that encompasses interactions and activities in personal, occupational, and recreational contexts. The biological underpinnings of psychosocial impairment are unclear. Few studies have specifically examined psychosocial functioning using brain activation and neuropsychological tasks.

The present study analyzed the association between psychosocial functioning and brain activation during higher cognitive functions in euthymic BD patients. We hypothesized that euthymic BD patients would exhibit different cortical activation during a verbal fluency task, but not during lower motor or sensory tasks, compared to healthy controls. More specifically, euthymic BD patients would have a more extended pattern of brain activation compared to the controls to achieve similar behavioral performance, reflecting insufficient processing in the anterior cingulate cortex (ACC) and left lateral PFC, which have been consistently reported to be activated during verbal fluency tasks. In addition, psychosocial functioning, known to be impaired in BD even during remission, was hypothesized to be associated with ACC and left lateral PFC activation during the task.

METHODS

Participants

The present study included 10 patients who met DSM-IV criteria for bipolar I disorder and 10 healthy volunteers with no history of neurological or psychiatric illness. Participants were all Japanese, and pairwise matched for age, sex, and years of education (Table 1). Healthy volunteers were required to be free of any recent use of any psychotropic medication. All participants were right-handed as assessed on the Edinburgh Handedness Inventory.[16] All patients were outpatients at the Hiroshima University Medical Hos-

Table 1. Demographics and behavioral performance

	BD patients mean ± SD	Normal controls mean ± SD	
n	10	10	
Gender (M/F)	4/6	4/6	
Age (years)	48.4 ± 8.8	54.6 ± 5.6	n.s. ($P = 0.12$)
Years of education	14.1 ± 2.0	13.5 ± 2.3	n.s. ($P = 0.38$)
Age at onset of first episode	35.0 ± 13.9		
Duration of illness (years)	13.6 ± 8.1		
HRSD score	2.8 ± 2.0	1.2 ± 1.1	n.s. ($P = 0.07$)
YMRS score	0.3 ± 0.6	0.1 ± 0.3	n.s. ($P = 0.50$)
GAF score	78.7 ± 7.9	90.8 ± 2.3	BD < controls ($P = 0.012$)
No. episodes	13.8 ± 14.9		
Mania	6.3 ± 5.8		
Depression	7.6 ± 9.4		
No. hospitalizations	2.7 ± 2.6		
Word fluency task	11.6 ± 4.2	10.2 ± 3.6	n.s. ($P = 0.52$)

BD, bipolar disorder; GAF, Global Assessment of Functioning; HRSD, Hamilton Rating Scale for Depression; YMRS, Young Mania Rating Scale.

pital. They had been diagnosed by two senior psychiatrists (including Y.O.) as having bipolar I disorder, using the Mini-International Neuropsychiatric Interview (MINI)[17] according to DSM-IV criteria. Healthy participants were recruited via advertisements. Exclusion criteria for both groups were current or past history of any psychiatric illness (other than BD for patients), organic disorder of the central nervous system, or a serious physical disorder, and standard MRI contraindications.[18] General functioning was assessed with the Global Assessment of Functioning (GAF) scale.[19] The GAF scale was administered by trained clinicians focusing on functioning instead of symptoms, and the assessment of the patients was based on individual interviews with the patients and with one of their family members.

Euthymia was defined as a score <7 on both the 17-item Hamilton Rating Scale for Depression (HRSD)[20] and the Young Mania Rating Scale (YMRS)[21] at the initial assessment and on the day of fMRI. All patients were euthymic for >1 month prior to scanning. All patients were receiving maintenance therapy at the time of the study. They had a stable pharmacotherapeutic regimen for a minimum of 6 weeks prior to study entry. Patients with BD were medicated as follows: lithium carbonate (400–800 mg) was taken by nine patients, sodium valproate (400–1600 mg) by four patients, olanzapine (2.5–20 mg) by two patients, and quetiapine (200 mg) by one patient. No patients were taking any antidepressants. Of the 10 BD patients, seven were taking two or more medications.

The study was conducted under a protocol approved by the Ethics Committee of the Hiroshima University School of Medicine. All participants provided written informed consent prior to their participation.

Experimental paradigm

We used a periodic design involving the presentation of a baseline condition for 30 s followed by an activation condition for 30 s. Each cycle was repeated three times over the course of 3 min.

Word fluency task

During the activation condition, participants were cued by the visual presentation of one of three letters (the Japanese phonetic characters that are pronounced 'sa', 'ta', and 'te') and asked to generate as many different words as they could beginning with that letter and to internally articulate the word. One of the three letters was presented every 3 s. During the baseline condition, participants were cued by visual presentation of the word 'yasumi' (which means 'rest') every 3 s and asked to internally articulate that word.

Before the scanning, all participants underwent a performance test outside of the scanner using the same design as described, with three different letters ('ka', 'na', and 'to'). On this test, participants were instructed to articulate the words audibly, not internally, and the number of different words generated was recorded.

Tapping task

During the activation condition, participants were cued by the visual presentation of the word 'yubiawase' (which means 'finger tapping') and asked to tap the thumb of the right hand to the forefinger, the middle finger, the third, and the little in order. This word was presented every 3 s. During the baseline condition, participants were cued by the visual presentation of a mosaic pattern every 3 s and asked only to watch the pattern without thinking about anything.

Visual task

During the activation condition, participants were cued by the visual presentation of a blinking checkered pattern every 3 s. During the baseline condition, they gazed at a fixed red cross in the center of the screen every 3 s. Throughout both conditions, the participants were asked only to watch the display without thinking about anything.

Image acquisition

Functional magnetic resonance imaging was performed using a 1.5-T Magnetom Symphony Maestro Class scanner (Siemens, Tokyo, Japan) at Kajikawa Hospital (Hiroshima, Japan). A time-course series of 64 volumes was acquired with T2*-weighted, gradient echo, echo planar imaging (EPI) sequences. Each volume consisted of 38 slices, and the slice thickness was 4 mm with no gap, and covered the entire cerebral and cerebellar cortices. The interval between two successive acquisitions of the same image (TR) was 3000 ms, the echo time (TE) was 48 ms, and the flip angle was 90°. The field of view (FOV) was 256 mm,

and the matrix size was 64 × 64, giving voxel dimensions of 4 × 4 × 4 mm. Scan acquisition was synchronized to the onset of the trial. After functional scanning, structural scans were acquired using a T1-weighted gradient echo pulse sequence (TR, 2050 ms; TE, 3.93 ms; flip angle, 15°; FOV, 256 mm; voxel dimensions, 1 × 1 × 1 mm), which facilitated localization.

Data analysis

Statistical analysis for demographic data and task performance of each group was performed using PASW 18.0 (Tokyo, Japan). To compare the age, years of the education, mood symptoms (HRSD and YMRS), GAF scores, and task performance of the word fluency task (WFT), Mann–Whitney *U*-test was used. Significance was defined as $P < 0.05$.

For image processing and statistical analysis we used statistical parametric mapping (SPM5) software (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 7.1 (Mathworks, Sherborn, MA, USA). The first (pre-task-period) and the last three (post-task-period) volumes were discarded, and the remaining 60 volumes were used for statistical analysis. Images were corrected for motion and realigned with the first scan of the session as a reference. T1 anatomical images were registered to the first functional image in each subject and aligned to a standard stereotaxic space, using the Montreal Neurological Institute (MNI) T1 template. The calculated non-linear transformation was applied to all functional images for spatial normalization. Finally, the functional images were smoothed with an 8-mm full-width, half-maximum Gaussian filter.

Using group analyses according to a random effect model that allowed inference to the general population,[22] we identified brain regions that showed significant responses during (i) word generation compared with word repetition; (ii) finger tapping compared with looking at a mosaic pattern; and (iii) looking at a blinking checkered pattern compared with looking at a red cross.

The group analysis consisted of two levels. In the first level, the signal time course of each subject was modeled with a delayed box-car function convolved with a hemodynamic response function in the context of a general linear model. One contrast image per subject was created by contrasting word generation with word repetition. In the second step, these images were entered into a one-sample *t*-test and then a two-sample *t*-test. Activations were reported if they exceeded $P < 0.05$, corrected for whole-brain false discovery rate at the single-voxel level and cluster extent of ≥50 contiguous voxels. In addition, voxel-wise correlational analysis (linear regression implemented in SPM5) between the activation of the WFT and clinical variables (BD group only and whole group) was applied for the voxels masked by the positive effect of WFT (inclusive mask threshold set at $P < 0.05$ uncorrected). This means that correlation analysis was performed for the regions that were related to the WFT. We used a relatively liberal threshold of $P < 0.01$ at the voxel level and cluster size >25 for this analysis, and limited inspection to a priori hypothesized regions. These a priori regions included the ACC and lateral PFC, because these regions are typically of interest for cognitive function and pathophysiology of BD. We then extracted the activation of each peak voxel, and used scatter plots for the activation and clinical variable only for visualization purpose. The xyz coordinates used in the present study are from the MNI brain space. Labels for brain activation foci were obtained in Talairach coordinates, which were converted from MNI coordinates, using the Talairach Daemon software, which provides accuracy similar to that of neuroanatomical experts.[23] The areas identified as labeled areas by this software were then confirmed by comparison with activation maps overlaid on MNI-normalized structural MRI.

RESULTS

Demographics

As shown in Table 1, there were no statistically significant differences between the two groups in terms of gender, age, years of education, HRSD score, or YMRS score. BD patients had significantly lower GAF score than normal controls.

Behavioral performance

All participants were able to complete the three tasks. There was no significant group difference in offline verbal fluency performance (Table 1).

Neuroimaging data

As shown in Figure 1, for both groups all tasks produced robust activations within the cerebral regions

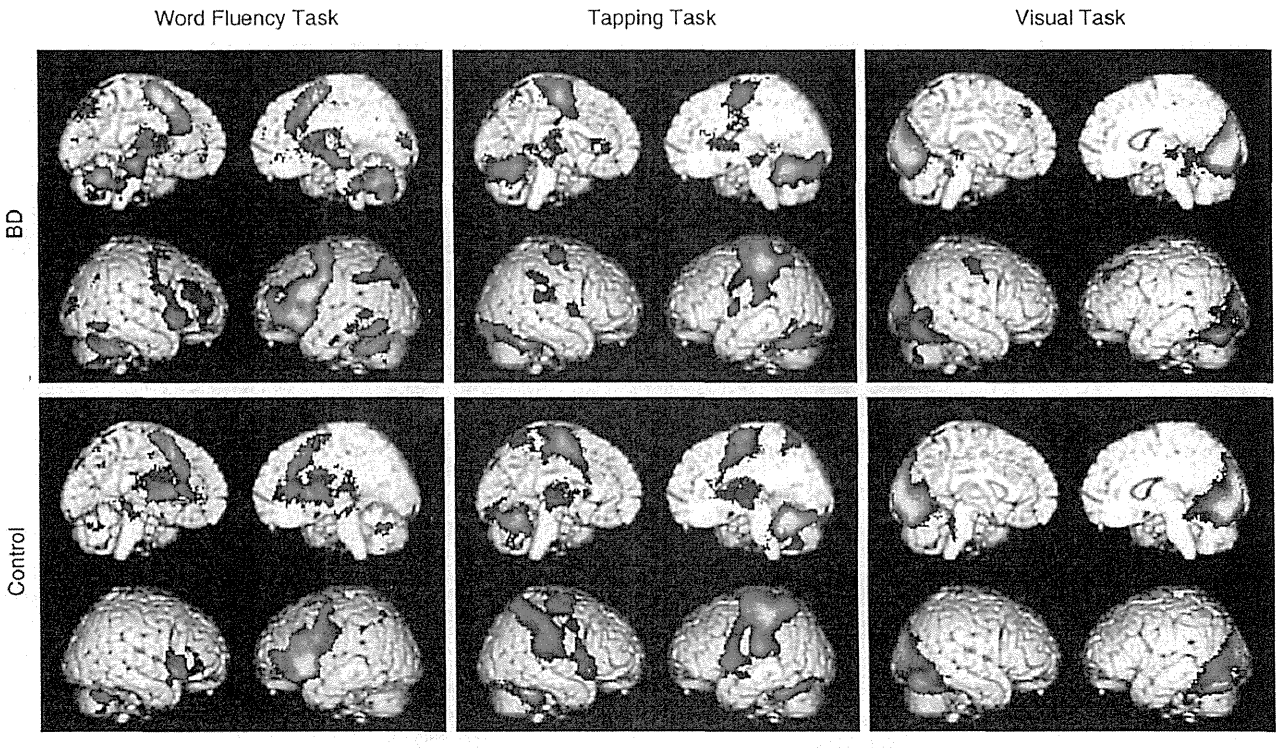

Figure 1. Brain activation during the verbal fluency task, tapping task and visual task in each group. For the word fluency task, healthy controls had activation in the lateral prefrontal cortex (PFC), anterior cingulate cortex (ACC), and cerebellum. Patients with euthymic bipolar disorder (BD) had a similar pattern of brain activation, with more widespread changes. Each group had significant activation in the left primary motor area for the tapping task, and in the bilateral primary visual area for the visual task (false discovery rate-corrected $P < 0.05$ and voxels >50).

that have previously been implicated in neuroimaging studies. A one-sample *t*-test for each group indicated significant activation in the left primary motor area for the tapping task, and in the bilateral primary visual area for the visual task. For the WFT, healthy controls had activation in the lateral PFC, ACC, and cerebellum. Patients with euthymic BD had a similar pattern of brain activation in these regions. Furthermore, additional regions including the bilateral precuneus were significantly activated in the BD patients.

Direct comparison between the two groups, using a two-sample *t*-test at each voxel of the brain activation, for the WFT showed that the BD patient group had significantly greater activation than the control group in the bilateral precuneus (left: [−14 −82 46], BA7, T = 5.21, voxels = 142; right: [16 −82 46], BA7, T = 5.21; voxels = 67). No other differences in significantly activated areas were seen for the control and the BD patient group (Fig. 2). There were no significant differences between the two groups for the visual task and the simple motor task.

Correlations in fMRI activation and clinical variables in BD patients

In the euthymic BD participants, activation in both the left ACC and the left lateral PFC were significantly positively correlated with GAF score (Fig. 3; ACC: [−10 38 24], BA32, T = 4.97, voxels = 75; left lateral PFC: [−50 14 4], BA45, T = 4.79, voxels = 34). No significant correlations were evident between brain activation and the offline performance or other clinical variables, such as age, years of education, onset age, duration of illness, number of episodes, or number of hospitalizations to the patient group. In addition, for all participants including the healthy controls, there was a significantly positive correlation between GAF score and activity in both brain areas (data not shown).

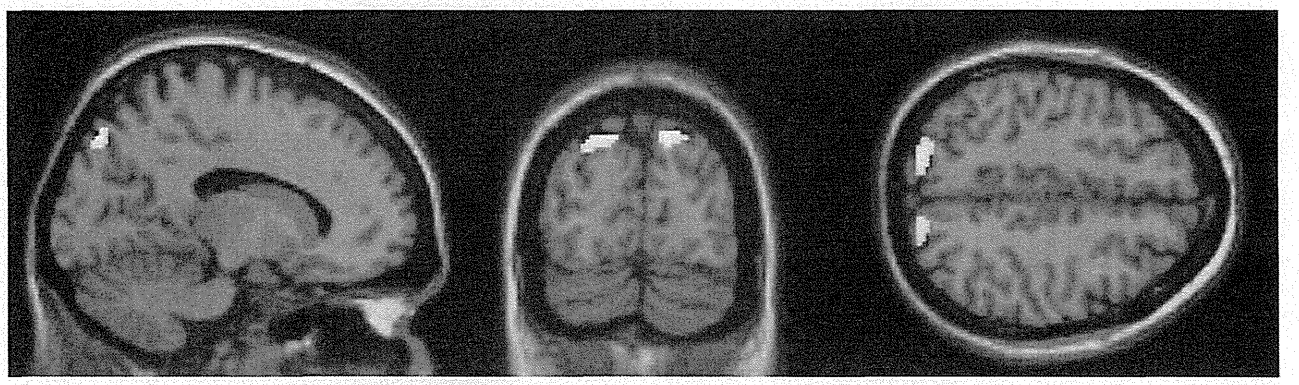

Figure 2. Word fluency task results. The bipolar disorder group had significantly greater activation than the control group in the bilateral precuneus (false discovery rate-corrected $P < 0.05$ and voxels >50).

DISCUSSION

The principal findings of this study are twofold. First, euthymic BD patients had greater activation in the bilateral precuneus compared to the controls, with similar behavioral performance during the verbal fluency task. There were no significant differences in patterns of brain activation between the two groups for the visual task and the simple motor task. Second, activation of both the left ACC and the left lateral PFC were significantly positively correlated with GAF score in the euthymic BD participants. There was no significant group difference, however, in the activations of the regions with which GAF score was correlated. This suggests that changes in the activation of these regions are associated with the current general functioning of BD patients, but are not the trait abnormality of BD. To our knowledge, this is the first study to show a clear relationship between functional brain activation and GAF in daily life in euthymic BD patients.

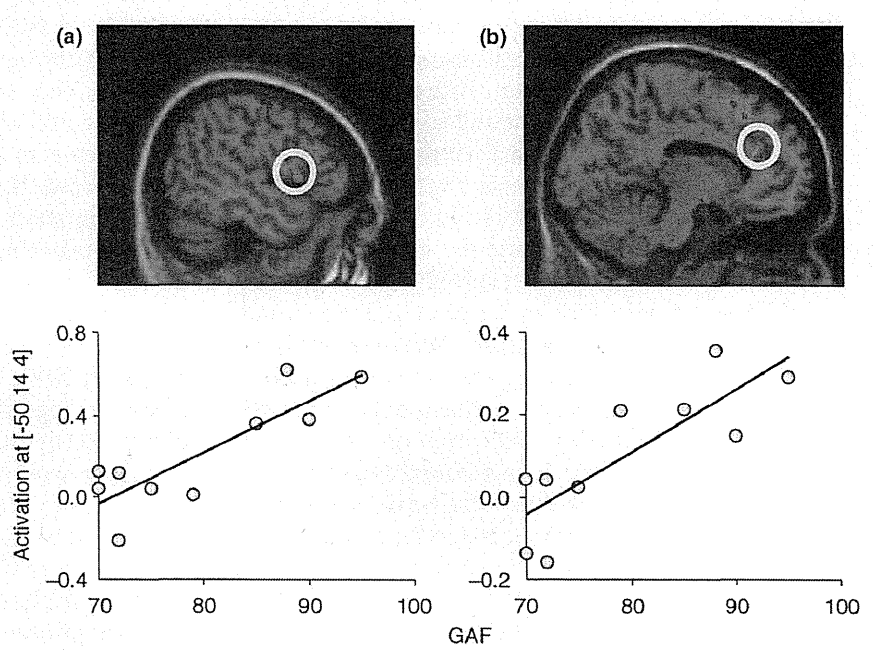

Figure 3. Correlations in brain activation and psychosocial functioning in bipolar disorder (BD) patients. In the BD group, activation in both the (a) left lateral prefrontal cortex (PFC) and (b) left anterior cingulate cortex (ACC) were significantly positively correlated with Global Assessment of Function (GAF) score (uncorrected $P < 0.01$ and voxels >25 masked by word fluency task activation $P < 0.05$).

Although the cross-sectional nature of the present study precludes definitive conclusions about a causal relationship, the present findings suggest that the poorer general psychosocial functioning found in euthymic BD patients may be attributable to attenuation of ACC and left lateral PFC activation during the higher cognitive task. Consistent with the present results at the behavioral level, verbal fluency has been reported to be a predictor of psychosocial functioning in BD patients.[24] Difficulty in retrieving verbal information may represent a serious problem for BD patients in their occupational functioning as well as in their interpersonal relationships. The ACC has been considered a key cortical area during the processing of cognitively demanding information. It has been shown to be associated with a number of functions including response selection, inhibition, vocalization and attention.[25] In contrast, the lateral PFC has been related to working memory, cognitive-set shifting, and planning.[26] Preceding neuroimaging studies have found alterations in the ACC–dorsolateral PFC system in both unipolar depressed and BD patients, putatively reflecting an abnormal interplay of monitoring and executive neurocognitive functions.[27]

One contradictory finding in the present study is that we failed to find a difference in the offline verbal fluency performance between the two groups, even though there was significant activation in the precuneus in euthymic BD patients compared to healthy controls. One explanation of this finding may be that the BD patients were able to produce similar numbers of words by activating more brain regions other than the usually activated areas such as the ACC, left lateral PFC, and cerebellum. This suggests that the patients used an alternative neural strategy to process verbal information to improve their performance. The present findings are in agreement with other studies that have found greater activation in different regions during cognitive tasks in patients with BD.[28,29] The precuneus has traditionally received little attention, mainly because of its hidden location and the virtual absence of focal lesion studies. Recent functional imaging findings in healthy individuals, however, suggest that the precuneus is a multimodal association area that is involved in episodic memory retrieval. It has been suggested that the prefrontal regions drive memory retrieval and that successful retrieval prompts the reactivation of engrams stored in the precuneus.[30] In the present study, BD patients had hyperactivation in the dorsoposterior portion of the precuneus near the parieto-occipital fissure during the verbal fluency task. This portion is functionally connected to adjacent visual cortical regions,[31] and related to visual and spatial attention. Therefore, the present results suggest that the BD patients required greater attention resources to perform the same task, possibly because of an inefficiency of the neural systems supporting verbal fluency performance. Interestingly, a considerable number of previous studies have indicated the differential recruitment of the precuneus between BD patients and controls, during various cognitive tasks. Greater activation in the precuneus of euthymic BD patients compared to controls during the verbal fluency task corroborates a previous study suggesting that BD patients have reduced deactivation in the precuneus, in comparison to healthy controls.[32] Significantly, studies using tasks other than the verbal fluency task have also suggested the diminished, or absent activity in the precuneus, as compared to healthy controls.[33,34] Although there is a lack of consistency in the direction of the effects observed across tasks, possibly because of differences in samples, imaging tasks, and analysis protocols among others, a study using voxel-based morphometry has also suggested that the precuneus is affected in BD.[35] It is suggested that the role of the precuneus in BD is an important issue for future investigation.

Several limitations must be considered when interpreting the present study. First, the overall number of participants is relatively small, which limits the generalizability of the study findings. The patient sample, however, consisted of well-diagnosed, stable individuals with chronic BD, and the sample size provided enough power to detect between-group differences. This sample size limitation is particularly true for correlation analysis, although the results for all participants are similar to those for BD patients. Nonetheless, this study provides some hypotheses for future research with larger samples. Second, the patients receiving medications were being treated with a variety of drug combinations, so that specific medication effects could not be determined given the number of patients available. Medications, however, may alter brain activation in regions that are associated with cognitive tasks, so the study of specific drug effects on cognition (and corresponding brain activation) in a larger patient sample is warranted. Third, the task performance data were obtained in an offline condition, but not in an online condition, because of the risk of verbal movement confounding the data. All participants, however, performed the same task using different phonemic characters outside of the scanner,

although there is no guarantee that the task performance at each session was the same. Fourth, the present paradigm had no sufficient baseline prior to the active block. This means that we cannot exclude the possibility that the early MRI data may include unstable magnetization. But we demonstrated that the activations on the WFT corresponded with the findings of previous studies. The effect of unstable magnetization seems to be small.

In conclusion, patients with euthymic BD had significant positive correlations between psychosocial functioning and activations in the ACC and the left lateral PFC during a verbal fluency task. Thus, the hidden cognitive disturbance, detected only on functional brain imaging with neuropsychological tests, is always present in BD, even though bipolar patients exhibit minimal affective symptoms during periods of euthymia. These results suggest that the precuneus may be sensitive to the impact of BD, and are also indicative of an association between the general functioning of euthymic BD patients and the function of the ACC and the lateral PFC. Further studies of brain activity using neuropsychological tasks as probes for psychosocial functioning in BD and other neuropsychiatric disorders are warranted.

ACKNOWLEDGMENTS

This study was supported by a Grand-in-aid for Science Research and 'Integrates research on neuropsychiatric disorders', the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant from Core Research for Evolutional Science and Technology (CREST) of Japan Science and Technology (JST). The authors do not have any conflict of interest.

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