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Author Contributions

Conceived and designed the experiments: TAK MW. Performed the experiments: TAK MW ST KI. Analyzed the data: MW. Contributed reagents/materials/analysis tools: TAK MW KH AM HU SK. Wrote the paper: TAK MW.

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Gamma Band Neural Synchronization Deficits for Auditory Steady State Responses in Bipolar Disorder Patients

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Abstract

Periodic auditory click stimulation has been reported to elicit an auditory steady state response (ASSR). The ASSR has been suggested to reflect the efficiency of γ-amino butyric acid (GABA) inhibitory interneuronal activity. Although a potential role for GABAergic dysfunction has been previously proposed, the role of neural synchronization in the ASSR in people with bipolar disorder (BD) has received little attention. In the current study, we investigated ASSRs to 20 Hz, 30 Hz, 40 Hz and 80 Hz click trains in BD patients. A total of 14 (4 males) BD patients and 25 (10 males) healthy controls participated in this study. ASSRs were obtained using whole-head 306-channel magnetoencephalography to calculate, ASSR power values and phase locking factors (PLF). BD patients exhibited significantly reduced mean ASSR power and PLF values bilaterally at frequencies of 30, 40, and 80 Hz ($p<0.05$ for these frequencies). At 20 Hz, bipolar patients showed no significant reduction in mean ASSR power and PLF values. There was a significant negative correlation between 80 Hz-ASSR-power values obtained from the right hemisphere and scores on the Hamilton Depression Rating Scale (rho = −0.86, $p = 0.0003$). The current study showed reduced low and high gamma band ASSR power and PLF bilaterally with no significant beta band ASSR reduction in BD patients. BD patients are characterized by deficits in gamma band oscillations, which may be associated with GABA inhibitory interneuronal activity dysfunction.

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Introduction

Periodic auditory click stimulation elicits an auditory steady state response (ASSR) that synchronizes to both the phase and frequency of the click stimulus. Several magnetoencephalography (MEG) studies have reported that source generators of ASSR are restricted to the primary auditory cortex [1], [2]. Neural circuitry functioning in the primary auditory cortex can be assessed using MEG-ASSR. The ASSR can reveal information about neural activity with respect to phase synchronization and response magnitude. The ASSR exhibits resonant frequencies in response to click trains at approximately 40 Hz and 80 Hz, although 40 Hz click trains produce responses of a larger magnitude [3].

Responses between 14 and 30 Hz are categorized as beta band activity, and rhythms >30 Hz are categorized as gamma band activity [4]. In addition, gamma band activity is subdivided into low (30–70 Hz) and high gamma band (>70 Hz) oscillations [5]. It has been suggested that the ASSR reflects the efficiency of γ-amino butyric acid (GABA) inhibitory interneuronal activity, which control the timing of pyramidal neurons in layer II/III of the cortex [6], [7]. Additionally, interactions between pyramidal neurons and inhibitory neurons have been found to produce emergent oscillations [8]. Emrich et al. proposed that GABAergic dysfunction plays a role in bipolar disorder (BD), based on the efficacy of valproate in the treatment of patients with this disorder [9]. Moreover, a post-mortem study of BD patients reported down-regulation in the expression of GABAergic genes (e.g., glutamic acid decarboxylase) [10]. Since ASSR is linked to GABA activity, investigations of ASSR are important in understanding BD.

In an MEG study of ASSR in BD, Maharajh et al. reported that patients exhibited a reduced right 40-Hz ASSR [11]. An electroencephalography (EEG) study by O'Donnell et al. reported reduced 20-, 30-, 40-, and 50-Hz ASSR in BD patients [12]. In addition, Rass et al. reported reduced ASSR power at 40 Hz and reduced ASSR synchronization at 40 Hz- and 50 Hz- stimulation in BD patients [13]. Studies of ASSR in schizophrenia (SZ) have consistently reported reduced gamma band ASSR [14–18]. For example, Light et al. reported that SZ patients exhibited reductions in both the evoked power and phase synchronization of ASSR to 30- and 40- Hz stimulation, but exhibited normal responses to 20- Hz stimulation [16]. Uhlhaas et al. suggested that

GABA is involved in the generation and synchronization of beta and gamma oscillations [4]. One computational modeling study (assuming that reduction of GABAergic interneurons increases the variability of GABA time constants) showed reduced 40 Hz responses and increased 20 Hz responses [19].

As discussed above, BD and SZ patients show similar patterns of ASSR deficits. Moreover, a post-mortem study reported a reduction in the numerical density of inhibitory interneurons in both BD and SZ [20]. Taken together, these findings indicate that neural circuitry dysfunction may exhibit similarities between these disorders at least to some extent. Recently, high gamma band oscillations have become a subject of increasing research interest [21], [22]. However, to our knowledge, only two studies have examined high gamma band ASSR (i.e., ASSR to 80 Hz click trains) in SZ [17], [18], with no studies of high gamma band ASSR in patients with BD. Overall, ASSR has received less attention in BD than in SZ research.

The current study used MEG to examine beta (ASSR to 20 Hz click trains), low (ASSR to 30 and 40 Hz click trains) and high gamma ASSR in BD patients. The present study was designed to test the hypothesis that BD patients exhibit reduced low and high gamma ASSR and no significant beta ASSR reduction.

Results

Demographic Characteristics

There were no significant group differences in age, handedness, self or parental SES or years of education (Table 1). There was no significant correlation between the dose of neuroleptic medication or lithium and ASSR power or PLF ($-0.48 \leq rho \leq 0.63$, $0.06 \leq p \leq 0.97$ for neuroleptics; $-0.65 \leq rho \leq 0.35$, $0.08 \leq p \leq 1.0$ for lithium). ASSR variables did not correlate significantly with valproate dosage, with the exception of significant negative correlations between right hemisphere 40 Hz-ASSR and the dosage (rho = −0.75, $p = 0.02$ for PLF; rho = −0.66, $p = 0.05$ for power).

To exclude the effects of transient gamma band responses [9], we also performed the analyses of the ASSR using a 200–500 ms window. The statistically significant results reported below remained the same. ASSR variables did not correlate significantly with demographic data or clinical scale scores ($-0.008 \leq rho \leq 0.54$, $0.07 \leq p \leq 0.93$) in either group, with the exception of a significant negative correlation between right hemisphere 80 Hz-ASSR-power and the Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH-D) scores (rho = −0.86, $p = 0.0003$) in participants with BD.

Mean ASSR Power

Figure 1 shows group-averaged time-frequency maps of ASSR power for each hemisphere. Values of mean±SD ASSR power are shown in Table 2. A repeated measures analysis of variance (ANOVA) demonstrated significant main effects of group ($F[1,37] = 7.0$, $p = 0.01$), frequency ($F[3,35] = 34.7$, $p < 0.0001$), and hemisphere ($F[1,37] = 10.9$, $p = 0.002$), and significant frequency-by-group ($F[3,35] = 3.4$, $p = 0.03$) and frequency-by-hemisphere ($F[3,35] = 3.4$, $p = 0.03$) interactions, with no other significant interactions ($0.50 \leq p \leq 0.64$). To delineate the significant frequency-by-group interaction, group differences were compared with *t*-tests using the average of both hemispheres for each frequency. Participants with BD showed significantly reduced ASSR power at 30-Hz ($t[37] = 3.1$, $p = 0.004$), 40-Hz ($t[37] = 2.6$, $p = 0.01$), and 80-Hz ($t[37] = 2.2$, $p = 0.03$), while no significant group differences were observed at 20-Hz ($t[37] = 0.38$, $p = 0.71$).

Table 1. Demographic and Clinical Characteristics of Participants.

	HC	BD	df	*t* or x^2	*p*
Sex, M/F, No	10/15	4/10	1	0.51	0.50
Age (years)	37.6±15.8	40.8±13.0	37	−0.68	0.50
Handedness	96.4±7.1	96.4±9.5	37	−0.01	0.99
SES	2.3±0.7	2.6±1.1	37	−0.85	0.40
Parental SES	2.8±1.0	3.1±1.1	37	−0.65	0.52
Education (years)	14.5±2.1	13.6±2.3	37	1.2	0.22
Symptom onset (years)		28.6±13.8			
Duration of illness (years)		11.6±9.9			
Medication dose (CPZ equiv., mg)		314±201			
YMRS		1.9±3.9			
SIGH-D		8.6±5.0			

Values are mean ± SD unless otherwise noted. HC: healthy controls, BD: patients with bipolar disorder,
SES = socioeconomic status, YMRS = Young Mania Rating Scale, SIGH-D = Structured Interview Guide for the Hamilton Depression Rating Scale.
Patients with BD were administered the following medications : N = 2 lithium & valproate; N = 1 lithium, quetiapine & zotepine; N = 1 lithium & quetiapine; N = 1 quetiapine, amoxapine & paroxetine; N = 1 valproate, amoxapine, trazodone & paroxetine; N = 1 valproate & quetiapine, N = 1 quetiapine & paroxetine; N = 1 *lithium, valproate, quetiapine & amitriptyline*; N = 1 *valproate & trazodone*; N = 1 lithium, valproate, olanzapine & risperidone; N = 1 valproate & quetiapine; N = 1 lithium, valproate & quetiapine; N = 1 lithium, quetiapine & levomepromazine.
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For 40 Hz harmonic response to 20 Hz stimulation, a repeated measures ANOVA demonstrated a significant main effect of hemisphere ($F[1,37] = 4.91$, $p = 0.033$), a trend-level significant group effect ($F[1,37] = 2.91$, $p = 0.096$), and no significant hemisphere-by-group interaction ($F[1,37] = 1.69$, $p = 0.20$), indicating trend-level reductions of 40 Hz harmonic powers to 20 Hz stimulation in BD patients.

Mean ASSR PLF

Figure 2 shows group averaged time-frequency maps of ASSR PLF for each hemisphere. The mean±SD of ASSR PLF values are shown in Table 3. A repeated measures ANOVA demonstrated significant main effects of group ($F[1,37] = 12.0$, $p = 0.001$), frequency ($F[3,35] = 49.8$, $p < 0.0001$), and hemisphere ($F[1,37] = 10.7$, $p = 0.002$), and significant frequency-by-group ($F[3,35] = 4.3$, $p = 0.02$) and frequency-by-hemisphere ($F[3,35] = 6.3$, $p = 0.002$) interactions, with no other significant interactions ($0.15 \leq p \leq 0.62$). To delineate the significant frequency-by-group interaction, group differences were compared with *t*-tests, using the average of both hemispheres for each frequency. Participants with BD exhibited significantly reduced ASSR PLF at 30-Hz ($t[37] = 3.1$, $p < 0.0001$), 40-Hz ($t[37] = 3.0$, $p = 0.005$), and 80-Hz ($t[37] = 2.3$, $p = 0.03$), while no significant group differences were observed for 20-Hz ($t[37] = 1.5$, $p = 0.17$).

For 40 Hz harmonic response to 20 Hz stimulation, a repeated measures ANOVA demonstrated no significant main effects of group ($F[1,37] = 2.33$, $p = 0.14$) or hemisphere ($F[1,37] = 3.36$, $p = 0.075$) and no significant hemisphere-by-group interaction ($F[1,37] = 1.21$, $p = 0.28$).

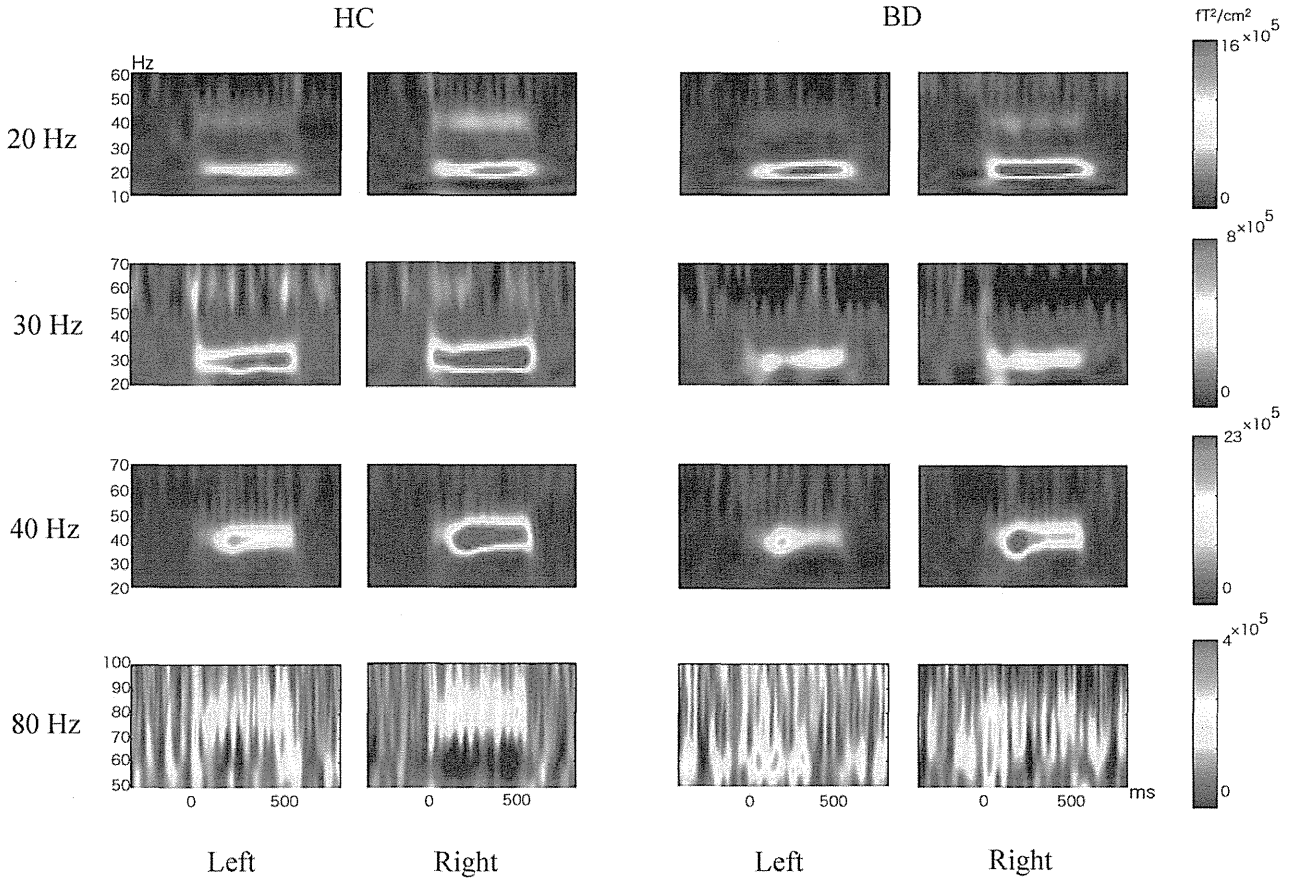

Figure 1. Group averaged time-frequency maps of ASSR-power for each hemisphere. The color scales signify ASSR-power. HC, healthy controls; BD, patients with bipolar disorder.
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Dipole Moments and Locations

Table 4 shows the group mean dipole moments for each group. A repeated measures ANOVA demonstrated significant main effects of group ($F\ [1,37] = 18.9$, $p = 0.03$), frequency ($F\ [3,35] = 53.8$, $p < 0.0001$), and hemisphere ($F\ [1,37] = 8.8$, $p = 0.005$), and significant frequency-by-group ($F\ [3,35] = 4.9$, $p = 0.003$) interactions, with no other significant interactions ($0.32 \leq p \leq 0.64$). To delineate the significant frequency-by-group interaction, group differences were compared with t-tests using the average of both hemispheres for each frequency. Participants with BD showed significantly reduced dipole moments at 30-Hz ($t\ [37] = 2.0$, $p = 0.05$), 40-Hz ($t\ [37] = 3.1$, $p = 0.003$), and 80-Hz ($t\ [37] = 2.0$, $p = 0.05$), while no significant group differences were observed for 20-Hz ($t\ [37] = -0.66$, $p = 0.51$).

With respect to dipole locations, a multivariate ANOVA (MANOVA) demonstrated no group effect and no interactions related to group, indicating that there were no significant group differences for dipole locations of the ASSR (see Table 5).

Table 2. Mean ASSR-power.

		HC (n = 25) (fT/cm)	BD (n = 14) (fT/cm)	df	t	p
20 Hz	Left	253.9±162.1	220.0±235.8	37	0.53	0.6
	Right	285.4±193.2	272.6±252.0	37	0.18	0.86
30 Hz	Left	264.4±176.0	152.5±80.1	37	2.2	0.03
	Right	318.3±187.0	166.8±112.2	37	3.2	0.003
40 Hz	Left	505.8±299.7	292.1±240.9	37	2.3	0.028
	Right	625.1±302.2	370.5±275.3	37	2.6	0.013
80 Hz	Left	76.6±73.5	46.1±35.7	37	1.7	0.09
	Right	96.5±84.8	48.9±39.7	37	2.4	0.023

Data are given as mean ± SD.
ASSR: auditory steady state response, HC: healthy controls, BD: patients with bipolar disorder.
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Discussion

The current study investigated the MEG-ASSR elicited by click trains of 20, 30, 40 and 80 Hz, and symptom-ASSR associations in patients with BD. The major findings in this study were: [1] BD patients exhibited bilaterally reduced mean ASSR power and PLF to 30-, 40- and 80- Hz stimulation, with no significant reduction to 20- Hz stimulation; [2] there was a significant negative correlation between right hemisphere 80 Hz-ASSR-power values and SIGH-D scores in patients with BD; [3] No significant group differences were observed in the dipole locations of ASSR.

To our knowledge, this is the first study to demonstrate both high and low gamma band ASSR deficits in patients with BD. Previous EEG studies reported reduced 20-, 30-, 40-, and 50-Hz

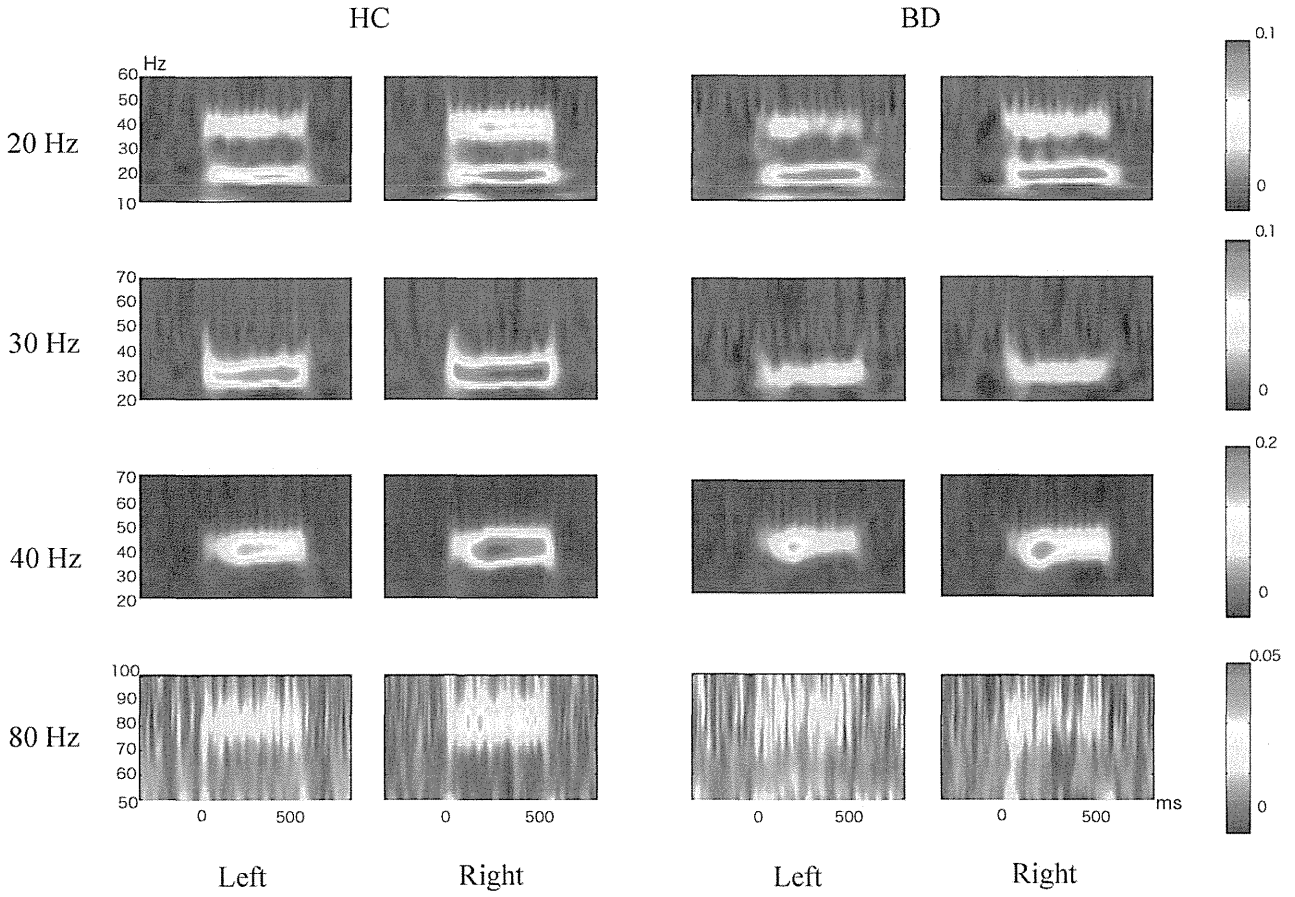

Figure 2. Group averaged time-frequency maps of ASSR-PLF for each hemisphere. The color scales signify ASSR-PLF value. HC, healthy controls; BD, patients with bipolar disorder.
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ASSR in people with BD [12] and reduced ASSR at 30 and 40 Hz in people with psychotic BD [23]. Rass et al. reported reduced ASSR power at 40 Hz and reduced ASSR synchronization to 40 Hz- and 50 Hz- stimulation in BD patients [13]. One MEG study reported that patients exhibited reduced right ASSR to 40 Hz- stimulation [11]. The present results partially support these previous findings. For the high gamma band, oscillations can be useful markers of cortical activity during a variety of cognitive tasks [21] and may reflect a fundamental aspect of temporal coding in cortical networks [22]. Additionally, different functions between beta and gamma oscillations have been suggested. Beta oscillations are related to sensory gating, attention and perception, and gamma oscillations are associated with memory and consciousness

Table 3. Mean ASSR PLF.

		HC (n=25)	BD (n=14)	df	*t*	*p*
20 Hz	Left	0.038±0.022	0.027±0.026	37	1.3	0.2
	Right	0.043±0.026	0.031±0.028	37	1.4	0.17
30 Hz	Left	0.044±0.031	0.022±0.011	37	3.1	0.004
	Right	0.053±0.03	0.023±0.012	37	4.3	<0.001
40 Hz	Left	0.091±0.051	0.052±0.038	37	2.5	0.018
	Right	0.11±0.046	0.063±0.042	37	3.3	0.002
80 Hz	Left	0.013±0.011	0.008±0.007	37	1.3	0.188
	Right	0.016±0.011	0.007±0.006	37	2.6	0.013

Data are given as mean ± SD.
ASSR: auditory steady state response, PLF: phase locking factor,
HC: healthy controls, BD: patients with bipolar disorder.
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Table 4. Dipole moments of the ASSR.

		HC (n=25) (nA/m)	BD (n=14) (nA/m)	df	*t*	*p*
20 Hz	Left	3.5±1.2	3.8±1.6	37	−0.55	0.59
	Right	3.6±1.4	3.9±1.3	37	−0.47	0.64
30 Hz	Left	2.9±1.1	2.5±1.1	37	1.1	0.29
	Right	3.9±1.4	2.9±0.9	37	2.4	0.02
40 Hz	Left	3.7±2.2	2.2±1.0	37	2.3	0.03
	Right	3.9±1.4	2.8±1.0	37	2.6	0.01
80 Hz	Left	1.3±0.8	1.1±0.3	37	1.0	0.31
	Right	1.6±0.9	1.2±0.4	37	1.5	0.14

Data are given as mean ± SD.
HC: healthy controls, BD: patients with bipolar disorder.
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Table 5. Dipole locations of the ASSR.

		Left (mm)			Right (mm)		
		x	y	z	x	y	z
20 Hz	HC (n = 25)	−45.6±4.9	6.6±12.7	61.6±9.0	49.1±7.3	9.5±9.5	61.4±12.8
	BD (n = 14)	−47.3±6.8	0.16±17.4	58.0±7.4	49.5±5.5	5.1±13.8	57.2±8.9
30 Hz	HC	−45.6±4.3	3.9±11.4	63.0±10.7	48.2±5.9	8.7±9.5	60.1±9.6
	BD	−45.8±5.1	3.0±16.4	60.9±8.0	47.5±7.8	5.7±14.7	59.4±8.0
40 Hz	HC	−47.0±5.2	4.3±13.0	60.9±10.9	50.6±11.8	9.3±10.5	58.3±14.3
	BD	−49.3±7.7	2.2±16.0	52.4±16.2	51.2±6.9	2.7±15.4	56.8±7.3
80 Hz	HC	−48.3±6.5	3.0±14.2	51.9±15.8	51.9±7.1	5.6±16.2	54.4±12.3
	BD	−46.7±5.4	−0.33±17.2	49.7±17.6	50.7±6.1	5.8±11.9	44.1±20.3

Data are given as mean ± SD. HC: healthy controls, BD: patients with bipolar disorder.
The zero point was the mid-point of the line connecting the bilateral preauricular points. The x-axis was the line from the left to the right with positive values toward the right, the y-axis was the postero-anterior line with positive values presented anteriorly, and the z-axis was the ventro-dorsal line with positive values located dorsally.
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as well as attention and perception [4]. Future studies should investigate the relationship between ASSRs and neural oscillatory activities during cognitive tasks in patients with BD, to clarify ASSR-cognitive related oscillations.

It has been suggested that GABAergic dysfunction plays a role in BD patients [9], [10]. The administration of mood stabilizers, such as valproate, carbamazepine, lithium, and lamotrigine, has been reported to increase GABA turnover in the mouse and rat brain [24–27]. In addition, valproate has been shown to increase plasma GABA levels in humans, suggesting that it enhances GABA activity in the human brain [28–30]. Recent *in vitro* studies have suggested that beta2 (20–30 Hz) oscillations are different from gamma oscillations in terms of generation. For instance, Cunningham et al. reported that the fast rhythmic bursting neurons in layer II/III play a crucial role in the generation of gamma oscillations [31]. GABAergic neurons have been reported to play a crucial role in the primary generation of gamma oscillations and their local synchronization [32]. In addition, direct electronic coupling through gap junctions between inhibitory neurons also contributes to the synchronization of gamma oscillations [33]. Both low and high gamma band oscillations can be generated by recurrent inhibition, but differ in their relationship to the spiking activity of parvalbumin-containing interneurons; in terms of their pharmacological modulation profiles as well as their layer specificity [5]. Conversely, an *in vitro* study by Roopun et al. reported that beta2 oscillations occurred in layer V pyramidal cells [34]. Moreover, this study indicated that beta2 oscillations are involved in gap junctional coupling and are independent of chemical synaptic transmission. The present study reported gamma band ASSR reduction and no significant reduction of beta band ASSR in BD patients, suggesting that BD might be characterized by hypofunction of GABA interneurons related to the fast rhythmic bursting neurons in layer II/III.

The present results revealed a significant negative correlation between right hemisphere 80 Hz-ASSR-power values and SIGH-D scores, indicating that BD patients with more severe depressive symptoms exhibited more reduced 80 Hz-ASSR power in the right hemisphere. However, this correlation should be confirmed in a larger sample. Rass et al. recorded 20-, 30-, 40-, and 50-Hz ASSRs in BD, and investigated associations between ASSRs and clinical status, cognitive function, and pharmacological treatment [13]. They reported that BD patients taking psychotropic medication exhibited decreased PLF relative to BD patients who had withdrawn from medication. In this study, mood state, psychotic features, cognitive performance, smoking, or history of substance use disorder were unrelated to ASSRs. Future studies that incorporate an assessment of patients before and after medication would be helpful in clarifying the associations between clinical symptoms and ASSR deficits in people with BD.

Reite et al. investigated ASSR source locations in people with BD. In normal control subjects the right hemisphere source was superior to the left, but no such hemisphere asymmetry was observed in BD patients [1]. However, the present results revealed no significant group differences in the dipole locations of ASSR. The heterogeneity of BD patients may account for this discrepancy. For example, the BD patients in the present study had never experienced psychotic symptoms and the sample was predominantly female, while Reite et al. examined 10 individuals with BD who had a history of psychosis and seven with no history of psychosis [1]. The ASSR of BD patients with a history of psychosis requires further investigation.

Several potential limitations of the current study should be considered. We were unable to exclude any treatment effects of mood stabilizers, neuroleptics or antidepressants on ASSR abnormalities in BD patients, and we found significant negative correlations between right hemisphere 40 Hz-ASSR and valproate dosage. Cross-sectional studies with more homogenous patient groups (drug-free vs medicated), as well as studies that assess participants before and after treatment with specific medications (thus controlling for health status) are required in future. Moreover, the effects of gender, and the ASSR of BD patients with a history of psychosis require further investigation.

Overall, the current study showed that BD patients exhibit reduced low and high gamma ASSR power and PLF bilaterally, with no significant beta band ASSR reduction. BD is characterized by gamma band ASSR deficits, which may be associated with dysfunctions of GABA inhibitory interneuronal activity.

Materials and Methods

Subjects

MEG data obtained from 14 (4 males, 10 females) individuals with BD and 25 (10 males, 15 females) healthy controls (HC) were analyzed in the present study. The data from 22 of the 25 HC participants were analyzed in our previous study [18]. The data

from 14 BD and 3 HC participants were newly recorded and analyzed for the present study. MEG recording was conducted between September 2007 and December 2009 for the HC group, and from July 2007 to May 2010 for the BD patients. We used the same recording equipment for both groups. All participants had normal hearing, were aged 20–60 years and were right-handed [assessed via Edinburgh Inventory [35]]. After being given a complete description of the study, all participants gave written informed consent in accord with the regulations of the Ethics Committee of the Graduate School of Medical Sciences, Kyushu University. Two senior clinical psychiatrists confirmed that all subjects had the ability to consent to participate in the examination. The exclusion criteria were: 1) neurological illness or major head trauma that would result in abnormal electroencephalography; 2) electroconvulsive therapy; 3) alcohol or drug dependence; 4) alcohol or drug abuse within the past five years; or 5) a verbal intelligence quotient below 75. HCs were screened using the Structured Clinical Interview (SCID), non-patient edition. No HCs exhibited any Axis-I psychiatric disorders, nor did their first-degree relatives.

All patients were recruited from Kyushu University Hospital and were diagnosed based on the SCID-DSM IV and medical records. No BD patients exhibited psychotic episodes. The patients were assessed using the Young Mania Rating Scale (YMRS) [36] and SIGH-D [37]. Demographic data for all subjects are presented in Table 1. Based on the criteria for depression [38] and euthymia [39], seven patients showed mild depression and seven were euthymic. Eight patients were receiving neuroleptic medication [typical neuroleptics (1/8 patients), atypical (7/8)], with a mean daily dose equivalent to 314±201 mg of chlorpromazine [40]. Regarding mood stabilizers, lithium was administered with a mean daily dose of 750±141 mg in eight BD patients, and valproate was administered with a mean daily dose of 844±445 mg in nine BD patients. The footnote in Table 1 lists the patients' medication.

Stimuli

The stimuli consisted of 1-msec clicking sounds, presented binaurally as trains of clicks for each stimulus frequency (20, 30, 40 and 80 Hz). The duration of each click train was 500 msec, and the intensity of the click trains was 80 dB sound pressure level. The inter-train interval was 500 msec. The mean number of presented click trains in one block was 313.9±105.7 for HC and 306.4±60.4 for BD, and there was no significant group difference ($t[37] = 0.24$, $p = 0.81$). The order of blocks was randomized across subjects.

Data Acquisition and Processing

The MEG signals were acquired using a whole-head, 306-channel sensor array (Vectorview; ELEKTA Neuromag, Helsinki, Finland). In this study, we analyzed MEG data recorded from 22-channel, planar-type gradiometers located at the sensor exhibiting the strongest response. This procedure was conducted for each hemisphere (Figure 3) based on our previous methods [18]. Prior to recording, four head position indicator (HPI) coils were attached to the scalp, and a three-dimensional (3D) digitizer was used to measure the anatomical landmarks of the head with respect to the HPI coils. The precise location of the head with respect to the sensor array was determined using the HPI coils. A band pass filter for recording was set to 0.01–330 Hz, and the sampling rate was 1 kHz. The subjects were instructed to keep their eyes open, remain attentive and listen to the trains of clicks presented through earphones. A spatio-temporal signal space separation (tSSS) method was applied off-line to the recorded raw data [41]. tSSS-reconstructed raw data with signal variations exceeding 4000 fT were excluded, and 200 responses were averaged for each type of stimulus as a result. The data were

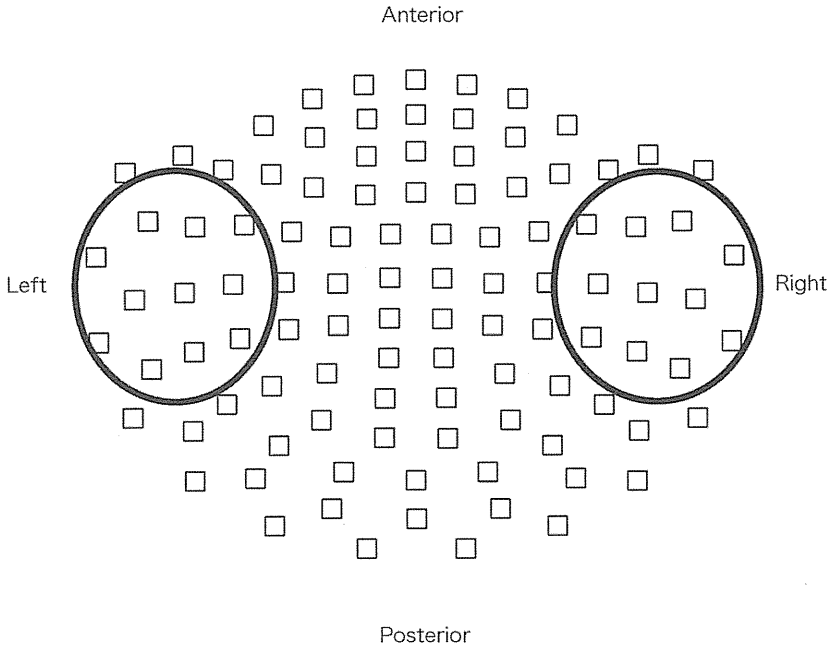

Figure 3. Layout of the measured channels. The MEG signals were acquired using a whole-head, 306-channel sensor array comprised of 102 identical triple-sensor elements. Each sensor consisted of two orthogonal planar-type gradiometers and one magnetometer. We used 11 sensors (a 22-channel orthogonal gradiometer) around the location that elicited the strongest response in each hemisphere. Circled squares indicate the sensors used for analysis.
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averaged with the following conditions: the analyzed period included the duration 400 ms before and 900 ms after stimulus onset.

Frequency Analysis

We used an estimation of the time-frequency energy based on the wavelet transform of the signal. The signal was convoluted by complex Morlet wavelets $\omega(t,f0)$ having a Gaussian shape with the wavelet being centered at the center frequency $f0$ and time t : $\omega(t,f0)=A\cdot\exp(-t^2/\sigma^2)\exp(2i\pi f0t)$, where $2\pi f0\sigma=7$ in 1-Hz steps. Wavelets were normalized so that their total energy was 1, with the normalization factor ^{A} equal to $(\sqrt{2\pi}\sigma)^{-1}$. We defined the squared modulus of the result of the convolution of a complex wavelet $\omega(t,f0)$ with the averaged responses $s(t)$: power $(t,f0)=|\omega(t,f0)\otimes s(t)|^2$ as the ASSR-power, where the symbol $\otimes$ indicates convolution. The square-root transform was applied to the ASSR-power for normalization. We also calculated the ASSR-phase-locking factor (PLF) using the following formula: PLF $(t,f0)=\frac{|\sum_i \frac{\omega(t,f)\otimes s(t)}{|\omega(t,f)\otimes s(t)|}|}{N}$. The PLF ranges from 0 (purely non-phase-locked activity) to 1 (strictly phase-locked activity). In calculating the power and PLF, we applied a baseline correction (from −200 to −100 msec). The mean power and PLF from 0–500 msec for each stimulus were averaged across 10-Hz bands.

Dipole Moments and Source Localization

The averaged responses were digitally filtered using a Butterworth filter (band pass; 15–25 Hz for the 20 Hz stimulation, 25–35 Hz for 30 Hz, 35–45 Hz for 40 Hz, and 75–85 Hz for 80 Hz). A single moving equivalent current dipole source model was applied, and dipole fits in each hemisphere were calculated by a least-squares fit. Single source dipole localization was performed for each time-point for 0–500 msec after stimulus onset. Only dipoles with goodness-of-fit criteria (>0.9) were chosen. The dipole locations were expressed by x, y, and z-coordinates.

Statistical Analysis

The mean ASSR powers and PLF were analyzed using a repeated measures ANOVA with group (BD or HC) as a between-subjects factor, and frequency (20, 30, 40 or 80 Hz) and hemisphere (left or right) as within-subjects factors. When significant interactions involving the group factor were identified, *post-hoc* analyses were conducted using t-tests. Additionally, 40 Hz harmonic ASSR powers and PLF to 20 Hz stimulation were analyzed using a repeated measures ANOVA with group as a between-subjects factor, and hemisphere as a within-subjects factor. For dipole locations, MANOVA was performed with group as a between-subjects factor, and frequency, hemisphere and axis (x, y or z) as within-subjects factors. Degrees of freedom were adjusted with the Huynh-Feldt epsilon for factors with more than two levels. Spearman's rho was used for correlation analyses. All results were considered significant at $p\leq 0.05$.

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Author Contributions

Conceived and designed the experiments: TO RT SK. Performed the experiments: YO NO IN. Analyzed the data: YO RT SH YH. Contributed reagents/materials/analysis tools: TU TM. Wrote the paper: YO TO SK.

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Reliability and Validity of the Japanese Version of BEMIB Modified for Patients With Bipolar Disorder: a Self-rating Scale for Medication Adherence

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ABSTRACT

Purpose: Accurate evaluation of medication adherence is important; however, no simple evaluation scale that is applicable to patients with bipolar disorder has been established in Japan. In this study, we prepared a modified Japanese version of a self-rating scale for medication adherence in the field of psychiatry, the Brief Evaluation of Medication Influences and Beliefs (BEMIB), and investigated its reliability and validity.
Methods: Forty-one patients with bipolar disorder who visited several facilities, including Nagoya University Hospital, from April 2006 to August 2006 and from April 2009 to July 2009 underwent medication adherence evaluations using the Japanese versions of BEMIB and the Drug Attitude Inventory-10 Questionnaire (DAI-10).
Results: The Cronbach α coefficient of the Japanese version of BEMIB was 0.73. Four-week test-retest reliability coefficients of each item and the BEMIB total score were 0.39-0.68 ($p < 0.05$) and the intra-class correlation coefficient was 0.63 (95% CI = 0.33-0.75, $p < 0.001$). In addition, a significant positive correlation was observed between the BEMIB and DAI-10 total scores (Pearson's correlation coefficient = 0.39, $p < 0.001$), showing that the concurrent validity was sufficient.
Discussion: The Japanese version of BEMIB modified for patients with bipolar disorder is sufficiently reliable and valid. We suggest that this simple evaluation scale of medication adherence in patients with bipolar disorder is applicable in routine medical practice.

Keywords: BEMIB, modified Japanese version, medication adherence, reliability, validity

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INTRODUCTION

Adherence requires a positive attitude on the part of the patients – accepting one's own disease and actively participating in deciding on a therapeutic policy. In a study in which physicians evaluated the adherence of patients by disease, adherence was worse in patients with chronic diseases, such as hypertension, diabetes and schizophrenia, than in people with headache or acute infections [1]. Specifically, adherence in mental disorders was lower than in patients with other chronic diseases. In psychiatric patients, medication adherence

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was associated with prognosis, indicating the importance of adherence for treatment success [2].

Medication adherence in the field of psychiatry has been investigated in many studies, including studies in schizophrenic patients [3,4]. In addition, the importance of medication adherence in improving prognosis in bipolar disorder has been attracting an increasing amount of attention [5,6]. Specifically, it has been reported that medication adherence in bipolar disorder patients is low (54-66%) [7-9], and that the recurrence rate is increased [10] and the incidence of suicidal behavior is increased five-fold [11] when medication adherence is poor.

To improve medication adherence, it first needs to be accurately evaluated. There are various evaluation methods, such as pill counting, measurement of the blood drug concentration, interview-based evaluations by physicians and self-rating scales. All of these methods have advantages and disadvantages [12]. Pill counting lacks reliability because the ingestion of drugs cannot be confirmed [13]. Measurement of the drug concentration in blood is a direct and objective method, but it has disadvantages, such as interindividual pharmacokinetic variability, interactions with food or other drugs, and favorable findings resulting from the re-initiation of medication for only a few days. Moreover, only a few drugs can be readily measured at clinical sites. Physician use of a structured interview that includes an evaluation scale, the Rating of Medication Influence (ROMI), has been reported [14]; however, this rating instrument is not used in routine clinical practice because of the training required for the person performing the interview, and because of the total amount of time needed to complete the interview. In a study in which the adherence of schizophrenic outpatients was evaluated by various methods, the proportion of patients considered nonadherent was 3% in self-reports, but 24% and 25% in physician evaluation and pill counting, respectively, and 52% in an evaluation method in which an electronic device, the Electronic Medication Monitor (EMM), was used. These results clearly show that there was extreme inconsistency among the methods [15].

Of the adherence evaluation methods, self-rating scales are simple and have been shown to be useful, although overestimation and recall bias are likely to occur in self-evaluation [11]. In a study mostly in bipolar disorder and schizophrenia, the adherence level estimated from the blood drug level was significantly correlated with the results obtained using self-evaluation [16]. In another study, the consistency with objective data (e.g., pill counting, plasma drug concentration, electronic measures) was higher than that of interview-based evaluation performed by physicians [17]. Although self-rating scales are useful, as described above, there are few instruments that have been translated into Japanese. Typical evaluation scales generally used in Western countries include the Brief Evaluation of Medication Influences and Beliefs (BEMIB) [18] and the Drug Attitude Inventory-10 Questionnaire (DAI-10) [19]. A Japanese version of DAI-10 [20] exists, and its application and rating system are simple; however, it also has the disadvantage that medication behavior is not evaluated. In other words, DAI-10 may evaluate compliance rather than adherence. BEMIB is another useful self-rating scale, prepared by Dolder and coauthors in 2004 (see reference 18, Fig. 1); its reliability and validity have been demonstrated in a study in sixty-three psychiatric outpatients in which adherence was investigated for six months based on refills. BEMIB is shorter than DAI-10, avoids the above disadvantages of DAI-10 and does not require training; however, a Japanese version is not yet available.

In the present study, we prepared a modified Japanese version of BEMIB, with the aim of establishing a self-rating scale in Japanese for the simple evaluation of medication adherence in patients with bipolar disorder, in whom medication adherence markedly influences the prognosis. We also investigated the reliability and validity of this scale.

SUBJECTS AND METHODS

Subjects

The subjects were 47 patients who were treated as outpatients at Nagoya University Hospital or its affiliated sites, Yagoto Hospital and Hinaga General Center for Mental Health, from April 2006 to August 2006 and from April 2009 to July 2009. The patients had received diagnoses of bipolar disorder type I or II using the bipolar disorder section of the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) [21], administered by expert psychiatrists. Patients with concurrent Axis I or II disorders (e.g., anxiety disorder, dementia and personality disorder) were excluded. Written informed consent was obtained from each participant after a full explanation of the study. This study was approved by the Ethics Committee of Nagoya University.

Methods

Preparation of Japanese version of BEMIB

After obtaining the consent of the author, the original BEMIB was carefully translated into Japanese by Japanese researchers including a bilingual psychiatrist whose first language is English. In order to apply BEMIB to patients with bipolar disorder, we translated the term "antipsychotic medication" to "psychotropic medication" in Japanese, with the permission of the original author. BEMIB is based on the modified health belief model [22], and its questions encompass all domains considered to influence medication adherence: benefits of treatment, risk of illness, costs of treatment, barriers to treatment and cues to act. BEMIB is composed of eight statements, each of which is evaluated using a 5-point Likert-type scale from 1 (completely disagree) to 5 (completely agree). A high score means favorable medication adherence, except for in statements 3 and 5, for which a low score represents high-level adherence.

Reliability

Evaluation of the modified Japanese version of BEMIB was repeated within four weeks to investigate test-retest reliability. To investigate the internal consistency reliability, the Cronbach α coefficient was determined. To avoid performance bias, the subjects were only told that this was a "study of an evaluation scale": there was no specific mention of "medication adherence."

Validity

To investigate the concurrent validity, the patients were simultaneously evaluated with the Japanese version of DAI-10 and with the modified Japanese version of BEMIB. The Japanese versions of the Beck Depression Inventory (BDI) [23] and the Altman Self-Rating Mania Scale (ASRM) [24] were used to evaluate depressive and manic symptoms, respectively. Since the clinical condition of a patient can change, the assessments performed using the Japanese versions of BEMIB, DAI-10, BDI and ASRM were repeated after twelve weeks. Pearson's correlation coefficients between the total scores of the modified Japanese version of BEMIB and the other scales were calculated. The scores at 0 and 12 weeks were used to analyze the correlation between the scales.

Data analysis

Statistical data are expressed as mean ± SD. Statistical analysis was performed using SPSS for Windows Version 20.0J. Significance was determined at the 0.05 level in all tests.

RESULTS

Characteristics of subjects

Of the 47 participants, one patient with major depressive disorder and five patients who could not complete one of the evaluation scales were excluded, leaving 41 patients who were ultimately included in the analysis. No patients dropped out during the study period. There were 22 males (54%) and 19 females (46%), and the mean age was 50.0 ± 12.3 years (27-73 years). The diagnosis was bipolar I disorder in 24 patients (59%) and bipolar II disorder in 17 patients (41%) (Tables 1, 2).

Table 1. Patient characteristics

Diagnosis	Bipolar I Disorder	23 (57%)
	Bipolar II Disorder	18 (43%)
Gender	Male	22 (54%)
	Female	19 (46%)
Age (years)		50.0 ± 12.4
Duration of illness (years)		16.1 ± 13.8
Mood stabilizers	Lithium	28 (68%)
	Valproate	18 (44%)
	Carbamazepine	1 (2%)
Dose of mood stabilizers (mg/day)	Lithium	596 ± 199
	Valproate	705 ± 280
	Carbamazepine	1000 ± 0

Table 2. Detailed patient characteristics

No.	Diagnosis	Gender	Age (y)	Duration of illness (m)	Mood stabilizers (mg/day)	Antipsychotics (mg/day)	Occupational Status
1	BP-I	m	32	14	Li 800		white-collar worker
2	BP-II	m	59	200	VPA 600		white-collar worker
3	BP-II	f	45	188	Li 400		housewife
4	BP-II	m	36	57	Li 600		blue-collar worker
5	BP-I	m	58	68	Li 800		retired
6	BP-II	f	42	75	VPA 600		housewife
7	BP-I	f	65	600	Li 400, VPA 400		housewife
8	BP-I	f	33	63	Li 600		white-collar worker
9	BP-I	f	59	79	Li 400	APZ 6	housewife
10	BP-I	m	51	21	Li 500		white-collar worker
11	BP-II	f	62	313	Li 400		housewife
12	BP-I	m	65	444	Li 400		white-collar worker
13	BP-I	f	37	210	Li 300, VPA 100	RIS 2	salesperson
14	BP-I	f	73	308	Li 400, VPA 600		housewife
15	BP-I	f	57	358	Li 600	RIS 1	housewife
16	BP-II	m	64	39	Li 300, VPA 600		farmer
17	BP-II	f	42	100	VPA 800		housewife
18	BP-II	f	48	220	Li 800		housewife
19	BP-I	m	42	136	Li 1000	QTP 400	retired
20	BP-I	m	61	103	Li 600	RIS 1	retired
21	BP-II	f	38	80	VPA 600		housewife
22	BP-II	f	71	43	VPA 400	QTP 50	housewife
23	BP-I	f	51	384	Li 800, VPA 1200		housewife
24	BP-II	m	35	156	VPA 600		care worker
25	BP-I	m	29	87	Li 600, VPA 1000		rehabilitation helper
26	BP-I	m	36	28	VPA 1000		blue-collar worker
27	BP-I	m	66	468	VPA 1200	QTP 750	retired
28	BP-I	m	37	21	VPA 600		blue-collar worker
29	BP-II	m	55	444	Li 400		blue-collar worker
30	BP-II	m	48	72	VPA 800		white-collar worker
31	BP-I	f	69	591	Li 400		retired
32	BP-I	m	54	312	Li 800	QTP 500	blue-collar worker
33	BP-I	f	58	366	Li 600		housewife
34	BP-I	m	45	197	CBZ 1000		unemployed
35	BP-II	f	27	43	VPA 1000	QTP 200	blue-collar worker
36	BP-II	m	43	147	Li 1000	QTP 200	white-collar worker
37	BP-II	m	53	75	Li 800	QTP 50	teacher
38	BP-I	f	51	67	VPA 600		housewife
39	BP-I	m	52	192	Li 800	OLZ 20	unemployed
40	BP-I	f	63	488	Li 600	RIS 2	housewife
41	BP-I	m	39	84	Li 600	QTP 200	unemployed

BP: Bipolar Disorder Li: Lithium VPA: Valproic Acid CBZ: Carbamazepine
APZ: Aripiprazole RIS: Risperidone QTP: Quetiapine

Results for each evaluation scale

A negative correlation was confirmed between the scores of statements 3 and 5 of the modified Japanese version of BEMIB and the total scores of the Japanese version of DAI-10. In contrast, we detected a positive correlation between the scores of all other statements of the modified Japanese version of BEMIB and the total scores of the Japanese version of DAI-10. As with the original version of BEMIB, it was considered adequate to invert the scores of two statements.

The mean total score of the modified Japanese version of BEMIB was 28.7 ± 4.6, and the mean total score of the Japanese version of DAI-10 was 4.89 ± 4.03.

The mean total scores of the modified Japanese ver-

sion of BDI on the first and second evaluations were 15.3 ± 12.1 and 14.0 ± 12.5, respectively, showing no significant difference between the two evaluations by paired t-test ($p = 0.47$). The mean total scores of the Japanese version of ASRM were 4.36 ± 3.26 and 3.95 ± 2.91, again showing no significant difference between the two evaluations by paired t-test ($p = 0.48$).

Reliability

The four-week test-retest reliability coefficients of each item and total score of BEMIB ranged from 0.39 to 0.68 ($p < 0.05$, Table 3) and the intra-class correlation coefficient (ANOVA-ICC) was 0.63 (95% confidence interval [CI] = 0.33-0.75, $p < 0.001$). The Cronbach α coefficient was 0.73. In the analysis of test-retest reliability, we surveyed the medical records of all the patients and confirmed that their moods were stable and they did not experience any mood episodes, and that they were treated on an outpatient basis during the four weeks.

Validity

Regarding the concurrent validity, the correlation level was determined by calculating Pearson's correlation coefficient. The total score of the modified Japanese version of BEMIB correlated significantly with the DAI-10 total score (correlation coefficient: 0.39; $p < 0.001$). However, no significant correlation was observed between the total scores of the modified Japanese version of BEMIB and BDI (correlation coefficient: -0.14; $p = 0.24$). In addition, there was no significant correlation between the total score of the modified Japanese version of BEMIB and the ASRM total score (correlation coefficient: 0.01, $p = 0.91$).

Table 3. Four-week test-retest reliability coefficients for the modified Japanese version of BEMIB

Item	r	p value
1	0.42	0.006
2	0.53	0.000
3	0.64	0.000
4	0.57	0.000
5	0.61	0.000
6	0.39	0.012
7	0.45	0.003
8	0.68	0.000
TOTAL	0.58	0.000

r: Pearson's correlation coefficient

DISCUSSION

Our study suggested that the modified Japanese version of BEMIB is sufficiently reliable. Using a four-week test-retest method, the score for each item and the total score of the modified Japanese version of BEMIB were significantly correlated; the intra-class correlation coefficient (ANOVA-ICC) was 0.63. According to a report describing the criteria of ANOVA-ICC in psychiatric clinical research [25], a value of 0.6 or higher is "satisfactory," suggesting that the Japanese version of BEMIB has satisfactory test-retest reliability. Moreover, the Cronbach α coefficient (0.73) was also acceptable, showing sufficient internal reliability.

According to our data, the modified Japanese version of BEMIB has satisfactory internal reliability and validity. Regarding the concurrent validity, the total scores of DAI-10 and the original BEMIB were significantly correlated (correlation coefficient: 0.55, $p < 0.001$) [18]. A significant positive correlation was also noted between the total scores of the Japanese version of DAI-10 and the modified Japanese version of BEMIB, suggesting that the concurrent validity was also sufficient. However, the correlation coefficient shown in our study is not as high as that shown by the original BEMIB. The DAI-10 lacks evaluation of medication behavior, and the Japanese version of BEMIB used in this study was modified for patients with bipolar disorder; these factors may account for the difference between the correlation coefficient shown in our study and that for the original BEMIB.

Only a few studies have focused on the association between changes in mood and medication adherence in patients with bipolar disorder [26]. The modified Japanese version of BEMIB was not significantly correlated with the Japanese version of BDI or ASRM. Depressive symptoms and manic symptoms may occur in patients with bipolar disorder, even though their medication adherence is good. If good adherence

becomes a habit, mood symptoms may not directly compromise adherence. Therefore, the scores of BDI and ASRM could change even when the BEMIB score remains stable. Moreover, higher adherence may lead to lower correlation between the scores of the modified Japanese version of BEMIB and BDI or ASRM. Actually, many previous studies have described poor insight as a factor influencing medication adherence, rather than symptom severity [27-29].

The modified Japanese version of BEMIB is advantageous in several respects compared with other scales designed for the evaluation of medication adherence. First, BEMIB does not require training and can be readily carried out in routine medical practice. Increases in total score indicate improved adherence. It is possible for patients to complete the scale by themselves while waiting for outpatient consultation; in our study, all patients could complete the scale within three minutes. Second, it can be combined with an education plan. Factors reducing medication adherence can be identified, facilitating investigation of a psychoeducational approach to improving adherence. It may also be possible to investigate changes in medication adherence caused by therapeutic interventions. Third, the modified Japanese version of BEMIB overcomes the problems of one of the few self-rating scales available in the Japanese language, DAI-10. The validity of DAI-10 is based on judgments made by physicians, and is disadvantageous in that medication behavior is not evaluated, but the modified Japanese version of BEMIB resolves this issue. For example, statement 4 of the modified Japanese version of BEMIB is: "I have a system (e.g., pill box, medication calendar, someone giving me my medication) that helps me remember to take my psychotropic medication." Finally, the statements are applicable to various treatment methods, and the scale is not limited only to pharmacological therapy.

Several limitations must be considered when interpreting the study findings. First, the sample size was relatively small. The sample size of the article describing the original study focused on BEMIB was also relatively small, with only 63 patients. As our study had only 41 patients, the statistical power may have been reduced, and consequently the possibility of a type 2 error cannot be completely ruled out. Second, since we investigated the reliability and validity only for bipolar disorder in this study, it was impossible to determine if the same results could be obtained in other psychiatric disorders. However, the statements in the modified Japanese version of BEMIB are not limited to bipolar disorder, and could potentially be applied to other psychiatric disorders. In this study, the original BEMIB was modified in translation from English to Japanese in order to evaluate medication adherence in patients with bipolar disorder. Back translation was not performed because the original BEMIB and its modified Japanese version are not identical in content. This means that the BEMIB modified for patients with bipolar disorder is available only in Japanese, not in English.

This study showed the usefulness of the modified Japanese version of BEMIB as an evaluation scale of medication adherence in the field of psychiatry using a sample that consisted of patients suffering from bipolar disorder.

CONCLUSIONS

This study demonstrated the sufficient reliability and validity of the modified Japanese version of BEMIB for patients with bipolar disorder. This is a useful tool well suited for the evaluation of medication adherence in patients with bipolar disorder, without training in routine medical practice.

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CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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SHORT COMMUNICATION

Differential effects of diazepam, tandospirone, and paroxetine on plasma brain-derived neurotrophic factor level under mental stress

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Objectives Serum brain-derived neurotrophic factor (BDNF) levels are reduced in depressed patients, and successful antidepressant treatment leads to increases in BDNF levels. However, little is known about how psychotropic drugs affect the mechanism of the human response to mental stress. We investigated the influence of psychotropic drugs on plasma BDNF levels under mental stress using a driving simulator (DS) task.
Methods Fourteen healthy male volunteers received one of four drugs, diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo, in a double-blind, crossover manner. Subjects were asked to perform the DS task 4 h post-dosing. Plasma BDNF levels were measured before and after the DS task.
Results Plasma BDNF levels under the placebo, diazepam, and tandospirone conditions significantly decreased after the DS task compared with before the task. Conversely, no significant differences in plasma BDNF levels were detected under the paroxetine condition.
Conclusion As these three psychotropic drugs have differential effects on plasma BDNF levels under mental stress after 4 h post-dosing, antidepressants, unlike anxiolytics, might have a prompt positive effect on the mental stress response.

KEY WORDS—antidepressant; anxiolytic drug; brain-derived neurotrophic factor; mental stress

INTRODUCTION

Stress is common in everyday life and is believed to affect happiness, health, and cognition (Caspi *et al.*, 2003). A role for brain-derived neurotrophic factor (BDNF) in the effects of stress and the response to antidepressant treatment is supported by studies demonstrating opposing regulation of this neurotrophic factor (Charmey, 2004). BDNF, the most abundant neurotrophin in the brain, enhances the growth and maintenance of several neuronal systems and serves as a neurotransmitter modulator (Shimizu *et al.*, 2003). BDNF is present in blood and can pass through the blood–brain barrier carried by a high-capacity, saturable transport system (Pan *et al.*, 1998). Although the source and function of blood BDNF remains unknown, recent reports have shown that more than 99% of blood BDNF proteins are stored in platelets and can be released in serum (Radka *et al.*, 1996) and that blood levels of BDNF might in part reflect BDNF levels in the brain (Karege *et al.*, 2002, Mitoma *et al.*, 2008).

The "neurotrophin hypothesis of depression" is based largely on two observations: a decrease in hippocampal BDNF levels is correlated with stress-induced depressive behavior, and antidepressant treatment enhances the expression of BDNF (Martinowich *et al.*, 2007). Recent studies suggested that serum BDNF levels are reduced in depression (Sen *et al.*, 2008, aan het Rot *et al.*, 2009). Antidepressants are thought to upregulate the expression of BDNF and its receptor and to promote adult neurogenesis, which might be the core pharmacological effect of antidepressants (Martinowich and Lu, 2008); successful antidepressant treatment leads to an increase in plasma BDNF levels (Lee and Kim, 2008).

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Stress can decrease the expression of BDNF in the hippocampus (Duman and Monteggia, 2006). However, little is known about how psychotropic drugs affect the human response to mental stress. In our previous study, we examined the effects of antidepressants and anxiolytic drugs using driving simulator (DS) tasks (Iwamoto *et al.*, 2008, Takahashi *et al.*, 2010). Here, we adapted the DS task as the psychological stressor in order to examine how mental stress influences plasma BDNF levels and to investigate the effect of psychotropic drugs on plasma BDNF levels under mental stress conditions.

MATERIAL AND METHODS

Fourteen healthy male volunteers (32–44 years old, mean ± SD, 37.2 ± 3.6 years) were included. All subjects had had a driving license for at least 10 years and regularly drove a car for a minimum of 5000 km per year. Health interviews and the Structured Clinical Interview for DSM-IV conducted at the time of the study indicated that none of the participants had any physical or psychiatric disorders. The study was approved by the Nagoya University Graduate School of Medicine and Nagoya University Hospital ethics review committee, and written informed consent was obtained from each subject prior to participation.

The schedule of this study is shown in Figure 1. The study was a double-blind, placebo-controlled, crossover study with four periods of treatment, each separated by a washout period of at least 7 days. Each subject was assigned to receive four treatments in a randomized, counterbalanced order set by laboratory personnel, who did not test subjects and analyze results. The random allocation sequence of each subject was concealed until the study termination. During each treatment period, the subjects received a single dose of each of the study drugs: diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo. The doses selected were based on general clinical recommendation for starting dose. All treatments were supplied in identical capsules for the double-blind design.

Each subject took one of the four drugs at 11:00 AM. The DS task was conducted 4 h after drug administration when the plasma concentration of paroxetine reaches its maximum (Doyle *et al.*, 1989, Ghose, 1989). Blood samples (10 mL) were collected in anticoagulant tubes before and after the DS task. Blood sample was immediately centrifuged at 1700 g for 10 min, and plasma sample was stored at −30 °C until used. Plasma BDNF levels were determined by enzyme-linked immunosorbent assay (Promega Co., Madison, WI, USA).

The car-following task in the DS task was used as the mental stressor. The details about this simulator (Toyota Central R&D Labs, Inc., Japan) are available elsewhere (Uchiyama *et al.*, 2003, Iwamoto *et al.*, 2008). The weighted average scores [adaptive weighted workload (AWWL)] (Miyake and Kumashiro, 1993) in the abridged Japanese version of the National Aeronautics and Space Administration Task Load Index (NASA-TLX) (Haga and Mizukami, 1996) was used to evaluate the mental stress of the car-following task. Seventeen healthy male volunteers completed the following two mental stress tasks using the DS in random order. One was a standard driving task, which required the subjects to drive the car freely on the road, and another was the car-following task that required the subjects to maintain a constant distance between the cars without the discretion of subjects. The time needed for the completion of both tasks is 5 min. The subjects were asked to rate the NASA-TLX after finishing each task, and the AWWL scores for each condition were calculated for subsequent analysis.

Statistical differences were determined with the paired *t*-test. Significance levels were set to 5% for all tests.

RESULTS

The AWWL scores for the car-following task condition were significantly higher than the normal driving task condition (mean ± SD: 55.2 ± 16.9 vs. 38.2 ± 20.8; $p < 0.01$).

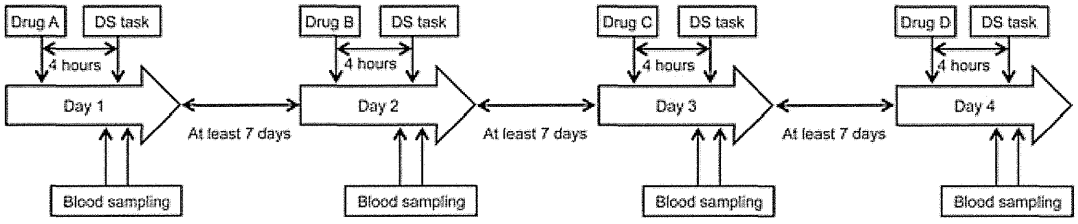

Figure 1. The figure shows the schedule of the study. Days 1, 2, 3, and 4 are treatment periods; each is separated by a washout period of at least 7 days. During each treatment period, the subjects received a single dose of one of the study drugs (drugs A, B, C, and D): diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo. Each subject took one of the four drugs at 11:00. The DS task was conducted 4 h after drug administration. Blood samples were collected before and after the DS task

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The effects of psychotropic drugs on plasma BDNF levels before and after the car-following task are shown in Figure 2. Under the placebo condition, plasma BDNF levels after the car-following task were significantly decreased compared with the plasma BDNF levels before the task (mean ± SD: 0.64 ± 0.31 vs. 0.34 ± 0.21, $p < 0.01$). We also found that under the diazepam and tandospirone conditions, plasma BDNF levels after the car-following task were significantly decreased compared with plasma BDNF levels observed before the task (mean ± SD: 0.49 ± 0.23 vs. 0.34 ± 0.21, $p < 0.05$ and mean ± SD: 0.59 ± 0.36 vs. 0.31 ± 0.14, $p < 0.01$, respectively). Conversely, these changes were not observed under the paroxetine condition (mean ± SD: 0.57 ± 0.27 vs. 0.79 ± 0.63, $p = 0.19$).

DISCUSSION

From the AWWL scores, we considered the car-following task as a mental stress condition. In the present study, we investigated the effect of psychotropic drugs on plasma BDNF levels under mental stress using a DS task as the stressor. Although the task associated with increased mental stress significantly decreased plasma BDNF levels under the diazepam, tandospirone, and placebo condition, the same effect was not observed under the paroxetine condition.

Regarding psychological stress, a previous study of healthy subjects demonstrated that levels of perceived mental stress in the workplace were inversely correlated with serum BDNF levels (Mitoma *et al.*, 2008). Both acute and chronic mental stress may reduce serum BDNF levels. According to these findings, mental stress might negatively affect stress-vulnerable depressed patients in whom serum BDNF levels are already decreased.

A previous report indicated that antidepressants could enhance BDNF gene expression by activating cyclic adenosine monophosphate response element binding protein (Martinowich and Lu, 2008). Furthermore, a recent study showed that antidepressants directly promote BDNF release from platelets in rats (Watanabe *et al.*, 2010). Considering that plasma BDNF levels did not significantly decrease under mental stress following acute administration of paroxetine in our result, paroxetine might promote short-term (several minutes) BDNF release from platelets in human models, although further examination would be needed.

Although anxiolytic drugs such as diazepam and tandospirone can relieve stress-related symptoms, there are no reports indicating that anxiolytic drugs influence plasma BDNF levels. One study showed that stimulation of the gamma-aminobutyric acid system (i.e., diazepam) in adult Wistar rats results in an immediate decrease in hippocampal BDNF mRNA levels (Zafra *et al.*, 1991). To our knowledge, the effects of diazepam on plasma BDNF levels have not been examined in humans. The present results suggest that benzodiazepine had no influence on plasma BDNF

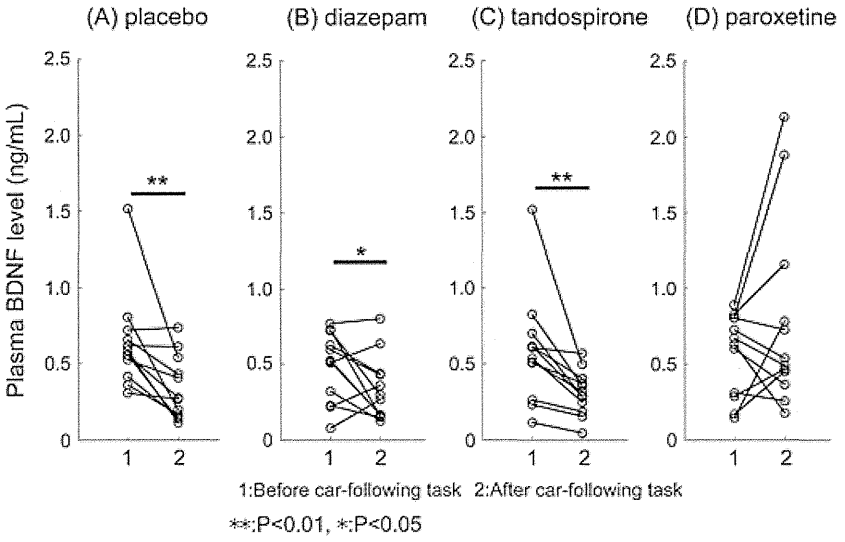

Figure 2. The effects of psychotropic drugs on plasma BDNF levels before and after the car-following task. Panel (A) shows the change in plasma BDNF levels during the placebo condition. Plasma BDNF levels after the car-following task are significantly decreased compared with levels before the task (p 0.01). Panels (B) and (C) show plasma BDNF levels following diazepam and tandospirone conditions. Plasma BDNF levels are significantly decreased after the car-following task compared with levels before ($p < 0.05$ and $p < 0.01$, respectively). Panel (D) shows plasma BDNF levels under the paroxetine condition. There is no significant difference in plasma BDNF levels before and after the car-following task ($p = 0.19$)

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