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Journal of Affective Disorders 136 (2012) 1047–1052

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Preliminary communication

More severe impairment of manual dexterity in bipolar disorder compared to unipolar major depression

Daimei Sasayama [a,b,*], Hiroaki Hori [a], Toshiya Teraishi [a], Kotaro Hattori [a], Miho Ota [a], Junko Matsuo [a], Yumiko Kawamoto [a], Yukiko Kinoshita [a], Miyako Hashikura [a], Naoji Amano [b], Teruhiko Higuchi [c], Hiroshi Kunugi [a,d]

[a] Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan
[b] Department of Psychiatry, Shinshu University School of Medicine, Matsumoto, 390-8621, Japan
[c] National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan
[d] CREST (Core Research of Evolutional Science & Technology), Japan Science and Technology Agency (JST) , Tokyo, 102-0075, Japan

ARTICLE INFO

Article history:
Received 22 September 2011
Received in revised form 26 October 2011
Accepted 14 November 2011
Available online 12 December 2011

Keywords:
Unipolar depression
Bipolar disorder
Dexterity
Lithium
Antipsychotics

ABSTRACT

Background: Mood disorders are associated with various neurocognitive deficits. However, few studies have reported the impairment of motor dexterity in unipolar depression and bipolar disorder. In the present study, manual dexterity was compared between unipolar major depression, bipolar disorder, and healthy controls.
Methods: Manual dexterity was assessed by the Purdue pegboard test in 98 patients with unipolar major depression, 48 euthymic or depressed patients with bipolar disorder, and 158 healthy controls, matched for age and gender.
Results: Compared to healthy controls, sum of the scores of right, left, and both hands subtests (R+L+B) was significantly lower in both patients with unipolar depression and bipolar disorder ($P=0.0034$ and $P<0.0001$, respectively). Furthermore, R+L+B was significantly lower in bipolar disorder compared to unipolar depression ($P=0.0016$). Lithium dose and chlorpromazine equivalent dose of antipsychotics were significantly negatively correlated with some of the subtest scores. On the other hand, depression severity did not significantly correlate with any of the subtest scores. Difference in R+L+B between unipolar depression and bipolar disorder remained statistically significant even after controlling for gender, age, lithium dose, and chlorpromazine equivalent dose ($P=0.0028$).
Limitations
Bipolar patients during manic episode were not included in the study.
Conclusions: Gross movement dexterity was impaired in both patients with unipolar depression and bipolar disorder. The severity of impairment was significantly greater in patients with bipolar disorder. The functional difference between unipolar and bipolar patients may suggest different pathological conditions between the two depressive disorders.

1. Introduction

Classifications of mood disorders are based on the polarity of episodes. However, similar depressive symptomatology between unipolar and bipolar disorders makes the differentiation difficult in depressed patients without a history of manic episode. Although several clinical characteristics such

* Corresponding author at: Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan. Tel.: +81 42 341 2712x5132; fax: +81 42 346 1744.
E-mail address: sasayama@shinshu-u.ac.jp (D. Sasayama).

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doi:10.1016/j.jad.2011.11.031

as hypersomnia and psychotic symptoms have been suggested to be helpful in distinguishing bipolar depression from unipolar depression (Forty et al., 2008; Mitchell et al., 2001), the lack of clear-cut clinical features distinguishing the two disorders has prompted researchers to seek genetic markers and endophenotypes. A few studies have found differences in personality profiles between unipolar and bipolar depression (Akiskal et al., 2006; Mendlowicz et al., 2005; Nowakowska et al., 2005; Sasayama et al., 2011). Recent evidence suggests that several neurocognitive deficits may also serve as endophenotypes of bipolar disorder (Bora et al., 2009; Langenecker et al., 2010). However, neurocognitive impairment is observed in unipolar depression as well (Han et al., in press; Schrijvers et al., 2009), and thus, whether there are characteristic neurocognitive deficits in bipolar disorder needs to be investigated.

Impaired dexterity is one of the neurocognitive phenotypes reported in patients with bipolar disorder (Langenecker et al., 2010; Wilder-Willis et al., 2001). On the other hand, some studies have also reported impaired fine motor movement in unipolar depression compared to healthy controls (Pier et al., 2004b; Swann et al., 1999). However, it remains to be elucidated whether the factors affecting dexterity and the severity of the impairment are similar in unipolar and bipolar disorder. In the present study, the Purdue pegboard test (Tiffin and Asher, 1948) was used to assess the manual dexterity in unipolar depression and bipolar disorder. The influence of depression severity and antipsychotics and lithium medications on dexterity was also examined.

2. Methods

2.1. Subjects

Subjects were 98 patients with unipolar major depressive disorder (50 patients with recurrent depression), 48 euthymic or depressive patients with bipolar disorder (8 patients with bipolar I and 40 with bipolar II disorder), and 158 healthy volunteers, matched for gender and age distributions. Participants were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in local free magazines, website announcement, notices posted in the hospital, flyers, and word of mouth. Only self-reported right-handed subjects were included in the study. Consensus diagnoses by at least two research psychiatrists were made according to the DSM-IV criteria (American Psychiatric Association, 1994) for unipolar major depressive disorder and bipolar disorder for enrollment in the study. Healthy participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist, and only those who demonstrated no history of psychiatric illness or contact to psychiatric services were enrolled as healthy controls. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence, or substance abuse. All subjects were biologically unrelated Japanese individuals who resided in the Western part of Tokyo. Written informed consent was obtained from all subjects prior to their inclusion in the study and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Measures

2.2.1. Purdue pegboard test

All participants were administered the Purdue pegboard test (Model 32030, manufactured by Lafayette Instrument Company USA) for evaluation of manual dexterity. The pegboard contains two vertical arrays of 25 holes in which pegs are placed one hand at a time and then with both hands simultaneously under timed conditions (30 s per trial). Scores for these measures (right, left, and both hands subtests) were derived for each trial according to how many pegs were placed within the time limit. The sum of the right, left, and both hands subtest scores (R + L + B) was used as the representation of gross dexterity of the fingers, hands, and arms. Fine fingertip dexterity was assessed by the assembly subtest, which involves using both hands alternately to construct assemblies consisting of a pin, a washer, a collar and another washer. This subtest requires participants to complete as many assemblies as possible within 60 s. The total number of pieces assembled was recorded as the score of the assembly subtest.

2.2.2. Handgrip force

Handgrip force was measured using a digital handgrip dynamometer (T.K.K.5401; Takei Co., Tokyo, Japan) to record the muscle strength of each hand. Participants were instructed to exert maximum grip force while standing upright, keeping their active arm stretched down vertically close to the body. The average of the two trials for each hand was defined as the maximal handgrip force.

2.2.3. Hamilton Depression Rating Scale

Depressive symptoms were assessed by an experienced research psychiatrist using the Japanese version of the GRID Hamilton Depression Rating Scale, 17-item version (HDRS) (Hamilton, 1967), which has been demonstrated to show excellent inter-rater reliability (Tabuse et al., 2007).

2.3. Statistical analyses

Statistical differences of demographic data among groups were evaluated by the chi-squared test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Student t-test was used for the post hoc analysis and for the comparisons of clinical variables between unipolar and bipolar patients. Due to the non-normal distribution of the Purdue pegboard scores and handgrip force, these data were compared between the three diagnostic groups using Kruskal–Wallis test, and thereafter, pairwise comparisons between two groups were done using Mann–Whitney test. Because antipsychotics and lithium, which are often prescribed for bipolar disorder, could cause tremors that may impair manual dexterity, correlations of the pegboard scores with the dose of these medications as well as with age, gender, antidepressant and anxiolytic prescription status, and HDRS scores were assessed using stepwise linear regression analysis (entry criteria $P<0.05$, removal criteria

$P>0.2$). Furthermore, analysis of covariance (ANCOVA) was performed to compare R+L+B scores between diagnostic groups while controlling for gender, age, antidepressant prescription status, lithium dose, and antipsychotic dose. R+L+B scores were squared before the ANCOVA to obtain normal distribution (Shapiro–Wilk test: $P>0.1$). The antipsychotic dose was calculated as chlorpromazine equivalent in mg/day according to published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999). Statistical significance was set at two-tailed $P<0.05$. Analyses were performed using the SPSS version 11.0 (SPSS Japan, Tokyo).

3. Results

Table 1 shows the demographic and clinical characteristics, Purdue pegboard scores, and handgrip force test results. Age distribution did not differ across the three diagnostic groups. Although the average years of education were highest in the controls, there was no significant difference between unipolar and bipolar patients. Over 35% of unipolar patients and 60% of bipolar patients were prescribed lithium and/or antipsychotics. Antidepressants and anxiolytics were also prescribed in 69% and 59% of unipolar patients and 54% and 63% of bipolar patients, respectively. Patients with unipolar depression and bipolar disorder did not differ significantly in age at onset or in HDRS scores. The mean score of every subtest of the Purdue Pegboard was highest in the control group and lowest in the bipolar disorder group. Post hoc pairwise comparisons with Bonferroni corrections revealed that R+L+B scores were significantly higher in control subjects compared to unipolar and bipolar disorders and were significantly lower in bipolar disorder compared to unipolar depression. Patients with bipolar disorder also scored significantly lower in assembly subtest scores compared to control subjects, although the difference with unipolar depression did not reach statistical significance. No significant

Table 1
Clinical characteristics and Purdue pegboard and handgrip force test results.

	Healthy controls (N=158)	Unipolar depression (N=98)	Bipolar disorder (N=48)	Statistical difference	Post hoc pairwise comparisons		
					Unipolar depression vs controls	Bipolar disorder vs controls	Unipolar depression vs bipolar disorder
Demographic characteristics							
Gender (male/female)	79/79	49/49	24/24	$\chi^2=0.00$, $P=1.00$			
Average age (years)	44.6 (14.8)	44.4 (13.5)	44.5 (14.5)	$F=0.004$, $P=1.00$			
Education years	15.4 (2.4)	14.4 (2.5)	14.6 (2.8)	$F=5.36$, $P=0.0052$	**$t=3.17$, $P=0.0017$**	$t=1.94$, $P=0.054$	$t=0.98$, $P=0.67$
Age at onset	na	35.0 (13.1)	31.7 (13.3)	$t=1.39$, $P=0.17$			
HDRS-17	na	10.9 (7.0)	11.5 (7.0)	$t=0.502$, $P=0.62$			
Medication status							
Antipsychotics without lithium (%)	0.0	25.1	25.0				
Antipsychotics with lithium (%)	0.0	6.6	27.1				
Lithium without antipsychotics (%)	0.0	4.0	8.3				
Other psychotropics only (%)	0.0	41.0	18.8				
No psychotropic medication (%)	100.0	13.2	20.8				
Purdue pegboard							
Right hand	14.9 (2.1)	13.9 (2.0)	13.0 (2.2)	$\chi^2=30.3$, $P<0.0001$	**$U=5751$, $P=0.0005$**	**$U=2022$, $P<0.0001$**	**$U=1719$, $P=0.0075$**
Left hand	14.1 (2.0)	13.3 (2.1)	11.9 (2.6)	$\chi^2=27.8$, $P<0.0001$	**$U=6256$, $P=0.0090$**	**$U=1978$, $P<0.0001$**	**$U=1614$, $P=0.0019$**
Both hands	11.6 (1.9)	11.3 (2.1)	10.1 (2.2)	$\chi^2=27.9$, $P=0.0001$	$U=7160$, $P=0.31$	**$U=2273$, $P<0.0001$**	**$U=1609$, $P=0.0017$**
Right+left+both hands	40.6 (5.1)	38.4 (5.5)	35.0 (6.2)	$\chi^2=31.6$, $P<0.0001$	**$U=6059$, $P=0.0034$**	**$U=1852$, $P<0.0001$**	**$U=1594$, $P=0.0016$**
Assembly	35.4 (8.0)	33.9 (8.6)	30.7 (9.3)	$\chi^2=12.4$, $P=0.0020$	$U=6576$, $P=0.043$	**$U=2617$, $P=0.0011$**	$U=1889$, $P=0.053$
Handgrip force test							
Right hand	33.1 (9.2)	31.9 (10.7)	31.2 (8.4)	$\chi^2=1.95$, $P=0.38$			
Left hand	31.3 (8.6)	29.6 (10.1)	29.5 (8.1)	$\chi^2=2.73$, $P=0.26$			

na: not applicable.
Bold indicates Bonferroni corrected significance of $P<0.017$ in the post hoc analysis.

difference was observed between groups in the results of the handgrip force test. Comparison between bipolar I and bipolar II disorders did not result in significant difference in the pegboard scores. However, each bipolar subtype showed significantly lower scores in R+L+B compared to healthy controls and unipolar depression (bipolar I vs controls: $P=0.0046$, bipolar II vs controls: $P<0.0001$, bipolar I vs unipolar: $P=0.045$, bipolar II vs unipolar: $P=0.0054$; Mann–Whitney test).

Table 2 shows the results of the stepwise linear regression analyses with R+L+B or assembly scores as the dependent variable. Age and gender, as well as lithium and antipsychotic chlorpromazine equivalent dose, antidepressant and anxiolytic prescription status (i.e., 0 = non-prescribed and 1 = prescribed), and HDRS scores in patient groups, were included as predictor variables. Age was negatively correlated with R+L+B and assembly scores in all diagnostic groups. Lithium dose showed significant positive correlation with assembly scores in the unipolar depression group. On the other hand, antipsychotic dose was significantly negatively correlated with R+L+B and assembly scores in bipolar disorder group and with R+L+B score in unipolar depression group. Significant negative correlation between lithium dose and R+L+B in patients with bipolar disorder was also observed.

Table 3 shows the results of the ANCOVA comparing square-transformed R+L+B scores between diagnostic groups. Each pairwise comparison yielded a statistically significant result.

Table 2
The results of the stepwise regression analyses.

	Right + left + both			Assembly		
	β	t	P value	β	t	P value
Healthy controls						
Age	−0.14	−5.81	<0.0001	−0.24	−6.35	<0.0001
Gender	2.79	3.92	0.0001	2.71	2.38	0.018
Patients with unipolar depression						
Age	−0.10	−2.09	0.040	−0.25	−3.28	0.0015
Gender	na	na	na	na	na	na
Lithium dose	na	na	na	0.01	2.27	0.026
Antipsychotic (CP equivalent) dose	−0.01	−2.09	0.039	na	na	na
Antidepressant medication use	na	na	na	na	na	na
Anxiolytic medication use	na	na	na	na	na	na
HDRS score	na	na	na	na	na	na
Patients with bipolar disorder						
Age	−0.19	−3.68	0.0007	−0.36	−4.51	<0.0001
Gender	na	na	na	na	na	na
Lithium dose	−0.01	−2.27	0.028	na	na	na
Antipsychotic (CP equivalent) dose	−0.02	−3.01	0.0045	−0.02	−2.56	0.014
Antidepressant medication use	na	na	na	na	na	na
Anxiolytic medication use	na	na	na	na	na	na
HDRS score	na	na	na	na	na	na

CP: chlorpromazine; HDRS: Hamilton depression rating scale; na: not applicable (not included in the stepwise model).

Table 3
The ANCOVA pairwise comparisons of the transformed R+L+B scores of the Purdue pegboard between unipolar and bipolar patients and healthy controls.

	Unipolar depression vs controls		Bipolar disorder vs controls		Unipolar depression vs bipolar disorder	
	F value	P value	F value	P value	F value	P value
Intercept	257.8	**<0.0001**	185.7	**<0.0001**	111.2	**<0.0001**
Gender	21.0	**<0.0001**	15.0	**0.0001**	5.1	0.026
Age	33.2	**<0.0001**	42.0	**<0.0001**	9.1	**0.0030**
Lithium dose	0.0	0.87	1.8	0.18	1.0	0.32
Antipsychotic (CP equivalent) dose	4.6	0.032	8.2	**0.0046**	10.1	**0.0018**
Diagnosis	7.2	**0.0077**	15.4	**0.0001**	9.3	**0.0028**

ANCOVA was performed with the square-transformed R+L+B scores as the dependent variable, diagnosis as the independent variable, and gender, age, lithium dose, and chlorpromazine equivalent dose as covariates. Bold indicates Bonferroni corrected significance of $P<0.017$.
CP: chlorpromazine; ANCOVA: analysis of covariance.

4. Discussion

Comparison with healthy controls revealed that the gross movement dexterity assessed by the R+L+B score was impaired in both unipolar and bipolar disorder patients. Furthermore, the severity of impairment was significantly greater in patients with bipolar disorder compared to patients with unipolar depression. No significant difference in handgrip force across diagnostic groups suggested that poor performance in the pegboard test in patients groups was not due to reduced muscle strength. Antipsychotic medications had significant negative influence on the gross movement dexterity. However, the impairment of gross movement dexterity in unipolar and bipolar disorder patients remained significant even after controlling for the effects of antipsychotic and lithium medications. Fine fingertip dexterity assessed by the assembly subtest was significantly impaired in patients with bipolar disorder.

Previous studies reported fine motor dysfunction in bipolar patients even when they were euthymic (Langenecker et al., 2010; Wilder-Willis et al., 2001). Although the patients in the present study included those in depressive states, the depression severity assessed by HDRS was not significantly correlated with the outcome of the pegboard scores. Furthermore, patients with bipolar disorder showed more severely impaired dexterity compared to patients with unipolar depression, despite the similar severity of depressive symptoms. Therefore, our results also suggest that the motor dexterity in bipolar disorder patients is impaired regardless of the presence of depressive symptoms.

Some studies have also reported fine motor slowing in patients with unipolar depression (Pier et al., 2004a, b; Schrijvers et al., 2009), consistent with our results. However, studies comparing the fine motor function between unipolar and bipolar patients are scarce. Swann et al. (Swann et al., 1999) examined dexterity assessed by continuous tapping of the right index finger in patients with unipolar depression and bipolar disorder. Their results showed that depressed

patients with unipolar depression and bipolar disorder showed equally reduced tapping speed compared to healthy controls; however, bipolar disorder patients during manic state did not show significant difference compared to the controls. On the contrary, our results suggested that patients with bipolar disorder showed more severe impairment of motor dexterity compared to patients with unipolar depression irrespective of the severity of the depressive symptoms. The different results in the study by Swann et al. (Swann et al., 1999) may be due to the sample selection and the method of evaluating motor function. Participants of the study by Swann et al. were inpatients while our study included only outpatients with relatively low HDRS scores. Thus, more severe depressive symptoms may have influenced the dexterity test outcomes. Also, the use of Purdue pegboard allowed us to evaluate the gross movement dexterity of fingers, hands, and arms instead of the fine motor speed of a finger assessed by the finger tapping test.

The most interesting finding of the present study was that patients with bipolar disorder were more severely impaired in motor dexterity compared to unipolar patients with similar severity of depressive symptoms. Both bipolar I and bipolar II patients, despite the small number of patients with each subtype, showed significantly lower scores in R + L + B compared to unipolar depression. Although bipolar patients were more likely to be prescribed with antipsychotics and/or lithium, the difference between unipolar and bipolar depression remained statistically significant even when these medications were controlled for.

The functional difference strongly suggests different pathological conditions between the two disorders. Swann et al. (Swann et al., 1999) reported that the relationship between psychomotor impairment and catecholamine function may be stronger in bipolar depression than in unipolar depression. Thus, the severer impairment of dexterity observed in bipolar depression may be etiologically different from that of the unipolar depression. There are other possibilities that could explain the difference in impaired dexterity between unipolar and bipolar depression. First, some of the patients with unipolar depression in this study may go on to experience a manic/hypomanic episode and be rediagnosed as bipolar disorder. Such patients may have been the cause of decreased R + L + B scores in the unipolar depression group. Secondly, unipolar depression may lie on a continuum with bipolar disorder (Akiskal and Benazzi, 2006), and thus, may show slightly impaired dexterity compared to healthy controls. Future studies should assess the motor dexterity in bipolar spectrum conditions (Akiskal et al., 2000) to examine these possibilities.

Another finding from the study worth noting is that antipsychotic medication had significantly negative influence on motor dexterity, which was consistent with findings in a recent study of schizophrenic subjects (Sponheim et al., 2010). Physicians should keep in mind that antipsychotics, often prescribed for those with bipolar disorder as well as unipolar depression, may enhance the disability caused by the impairment of dexterity.

There are several limitations to this study. First, the cross-sectional design did not allow any definitive conclusions as to whether the impairment of the motor dexterity preceded or resulted from illness onset. Furthermore, some patients with unipolar depression in this study may be rediagnosed as bipolar disorder in the future, and thus follow-ups are necessary for accurate diagnosis. Secondly, the number of patients with bipolar disorder was small. Larger studies are needed to compare bipolar I and II disorders. Thirdly, as the patients were limited to those receiving outpatient treatments, our subjects might have been overrepresented by milder forms of illness. Moreover, we did not include bipolar patients during the manic episode. Further studies are necessary to determine whether dexterity is dependent on the phase of the disorder. Fourthly, the self-reported handedness was not verified using a validated hand preference questionnaire. A previous study has shown that non-right handedness is associated with soft bipolarity in mood disorders (Fasmer et al., 2008). Therefore, different rates of mixed-handed persons in each diagnostic group may have confounded the results of the pegboard test. However, since significant impairment of dexterity in bipolar disorder was observed in both the right hand and the left hand subtests, our conclusion that dexterity is impaired in bipolar disorder is not weakened by the possible inaccuracy of the handedness. Finally, the effects of medication could not be fully controlled due to the variability in types and doses. However, analyses examining the influence of antipsychotics and lithium on the outcome of the Purdue pegboard test indicated that these medications were not the only explanatory variable to the impaired dexterity.

In conclusion, we assessed manual motor dexterity in patients with unipolar depression and bipolar disorder and confirmed that both unipolar and bipolar patients were impaired in gross motor dexterity when compared to healthy controls. However, the severity of impairment was significantly greater in bipolar disorder compared to unipolar depression, despite the similar severity of depressive symptoms. The functional difference between unipolar and bipolar depression may suggest different pathological conditions between the two depressive disorders.

Role of funding source

Funding for this study was provided by Health and Labor Sciences Research Grants (Comprehensive Research on Disability, Health, and Welfare; H21-kokoro-002) (H.K.), JST, CREST (H.K.), and Intramural Research Grant (21-9) for Neurological and Psychiatric Disorders of NCNP. They had no further role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgment

The authors would like to thank the participants for taking part in the study.

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Psychopharmacology (2012) 219:1099–1109
DOI 10.1007/s00213-011-2440-z

ORIGINAL INVESTIGATION

Behavioral and molecular evidence for psychotropic effects in L-theanine

Chisato Wakabayashi · Tadahiro Numakawa · Midori Ninomiya · Shuichi Chiba · Hiroshi Kunugi

Received: 1 April 2011 / Accepted: 2 August 2011 / Published online: 23 August 2011

Abstract
Rationale L-Theanine (*N*-ethyl-L-glutamine) is an amino acid uniquely found in green tea and historically considered to be a relaxing agent. It is a glutamate derivative and has an affinity for glutamatergic receptors. However, its psychotropic effects remain unclear.
Objectives To elucidate effects of L-theanine on psychiatric disease-related behaviors in mice and its molecular basis focusing on brain-derived neurotrophic factor (BDNF) and *N*-methyl-D-aspartate (NMDA) receptor.
Methods We examined the effects of L-theanine on behaviors in mice by using the open-field test (OFT), forced swim test (FST), elevated plus-maze test (EPMT), and prepulse inhibition (PPI) of acoustic startle. By western blot analysis, we looked at the effect of L-theanine on the expression of BDNF and related proteins in the hippocampus and cerebral cortex. To determine whether L-theanine has agonistic action on the NMDA receptor, we performed Fluo-3 intracellular Ca^{2+} imaging in cultured cortical neurons.
Results Single administration of L-theanine significantly attenuated MK-801-induced deficits in PPI. Subchronic administration (3-week duration) of L-theanine significantly reduced immobility time in the FST and improved baseline PPI. Western blotting analysis showed increased expression of BDNF protein in the hippocampus after subchronic administration of L-theanine. In cultured cortical neurons, L-theanine significantly increased the intracellular Ca^{2+} concentration, and this increase was suppressed by competitive and non-competitive NMDA receptor antagonists (AP-5 and MK-801, respectively).
Conclusions Our results suggest that L-theanine has antipsychotic-like and possibly antidepressant-like effects. It exerts these effects, at least in part, through induction of BDNF in the hippocampus and the agonistic action of L-theanine on the NMDA receptor.

Keywords L-theanine · MK-801 (dizocilpine) · *N*-methyl D-aspartate (NMDA) receptor · Schizophrenia · Prepulse inhibition · Brain-derived neurotrophic factor (BDNF) · Behavior · Glutamate · Antidepressant

Abbreviations

OFT	Open-field test
PPI	Prepulse inhibition
FST	Forced swim test
EPMT	Elevated plus-maze test
NMDA	*N*-methyl-D-aspartate
GABA	Gamma-aminobutyric acid
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
BDNF	Brain-derived neurotrophic factor
PCP	Phencyclidine
AP-5	D-(−)-2-amino-5-phosphonopentanoic acid

Electronic supplementary material The online version of this article (doi:10.1007/s00213-011-2440-z) contains supplementary material, which is available to authorized users.

C. Wakabayashi · T. Numakawa · M. Ninomiya · S. Chiba · H. Kunugi (✉)
Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187-8502, Japan
e-mail: hkunugi@ncnp.go.jp

T. Numakawa · H. Kunugi
Core Research for Evolutional Science and Technology Program (CREST), Japan Science and Technology Agency (JST), Tokyo 102-0075, Japan

Introduction

L-Theanine was originally found in green tea and has historically been recognized as a relaxing agent (de Mejia et al. 2009; Nathan et al. 2006). Besides its similar chemical structure to glutamate, L-theanine shows micromolar affinities for kainate, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), and *N*-methyl D-aspartate (NMDA) glutamate receptors (Kakuda et al. 2002). Neurochemical studies suggest that L-theanine affects serotonin, dopamine, and γ-aminobutyric acid (GABA) levels in the brain (Yamada et al. 2007; Yokogoshi et al. 1998a, b). It has also been shown to exert neuroprotective effects (Kakuda 2002).

L-Theanine is known to exert relaxing effects; for example, it increases the alpha wave in the human electroencephalogram (Juneja et al. 1999; Nobre et al. 2008). It has also been shown to lower physiological stress responses, by reducing heart rate and salivary immunoglobulin A responses during an acute stress task (Kimura et al. 2007). However, whether L-theanine has anxiolytic, antidepressive, or antipsychotic effects remains unknown. To our knowledge, only one study has examined the effect of L-theanine on anxiety-like behavior in rats, by using standard tests such as the elevated plus-maze test (EPMT) (Heese et al. 2009) and yielding equivocal results. Regarding antidepressant-like effect, L-theanine showed antidepressant-like activity in the forced swim test (FST) and tail suspension test (TST) (Yin et al. 2011). A very recent clinical study showed that L-theanine relieved positive, activation, and anxiety symptoms in patients with schizophrenia and schizoaffective disorder (Ritsner et al. 2011). In addition, a combination of green tea extract and L-theanine improved memory and attention in individuals with mild cognitive impairment (Park et al. 2011). To our knowledge, however, no study has assessed whether L-theanine has the effects of antipsychotic-like behaviors by means of prepulse inhibition (PPI) test.

It has been widely accepted that deficits in PPI of acoustic startle indicate impairment of sensorimotor gating and occur in patients with schizophrenia (Braff et al. 2001; Kunugi et al. 2007). Competitive and non-competitive NMDA receptor antagonists (e.g., AP-5, phencyclidine, MK-801 [dizocilpine], and ketamine) produce robust deficits in PPI in rodents (Geyer 2006; Geyer et al. 2001; Kretschmer and Koch 1997; Mansbach and Geyer 1989). Animals that are administered such drugs are considered as models for schizophrenia (Furuta and Kunugi 2008). Because L-theanine binds to NMDA receptors (Kakuda et al. 2002), we considered it intriguing to examine the possible effects of L-theanine on PPI.

Many studies indicate that brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays important roles in neuronal survival and synaptic plasticity (Numakawa et al. 2002), via activation of TrkB, a high-affinity receptor for BDNF. Recently, we investigated the effects of BDNF on functions of hippocampal and cortical neurons (Kumamaru et al. 2008; Matsumoto et al. 2006; Numakawa et al. 2009). Many researchers have reported altered levels of BDNF in the postmortem brains, in the peripheral blood of patients with schizophrenia and those with mood disorders, and in the brains from animal models of depression and schizophrenia (reviewed in Duman and Monteggia 2006; Green et al. 2010; Kunugi et al. 2010; Pillai 2008).

The aims of the present study were threefold. First, we examined whether L-theanine has anxiolytic-like, antidepression-like, and antipsychotic-like effects in mice, by using a behavioral test battery, i.e., the open-field test (OFT), EPMT, FST, and PPI test. Second, we examined the possible effects of L-theanine on the levels of BDNF and its receptors and also on glutamate receptors in the mouse brain (i.e., the hippocampus and cerebral cortex). Third, to clarify whether L-theanine exerts agonistic or antagonistic effects on NMDA receptors, we examined whether L-theanine affects intracellular Ca^{2+} influx, and whether such effects are suppressed by NMDA receptor antagonists in cultured cortical neurons.

Materials and methods

Animals

C57BL/6 male mice aged 10–11 weeks old, weighting 25–28 g (for single administration) or aged 5 weeks old, weighting 19–21 g (for subchronic administration) at the beginning of experiments were purchased from SLC, Japan. Mice were housed under specific pathogen free (SPF) conditions in standard cages (16×22×14 cm; four or five mice per cage) with wood chips in a temperature (23–25°C)- and humidity (45–55%)-controlled environment with a 12/12-h modified dark/light cycle (light on at 16:30 P.M.). Food and water were available ad libitum. All behavioral experiments were performed in the behavioral experimentation room during the dark phase of the light cycle under a 30-lux lit condition. All experimental procedures were in accordance with the guidelines of the United State's National Institutes of Health (1996) (http://oacu.od.nih.gov/regs/guide/guide.pdf) and were approved by the institutional Animal Care and Use Committee.

Time schedules of drug administration, behavioral tests, and sample collection are described in Fig. 1.

Drugs

L-theanine, MK-801, AP-5, and fluoxetine hydrochloride were purchased from Sigma (St. Louis, MO, USA). Drugs

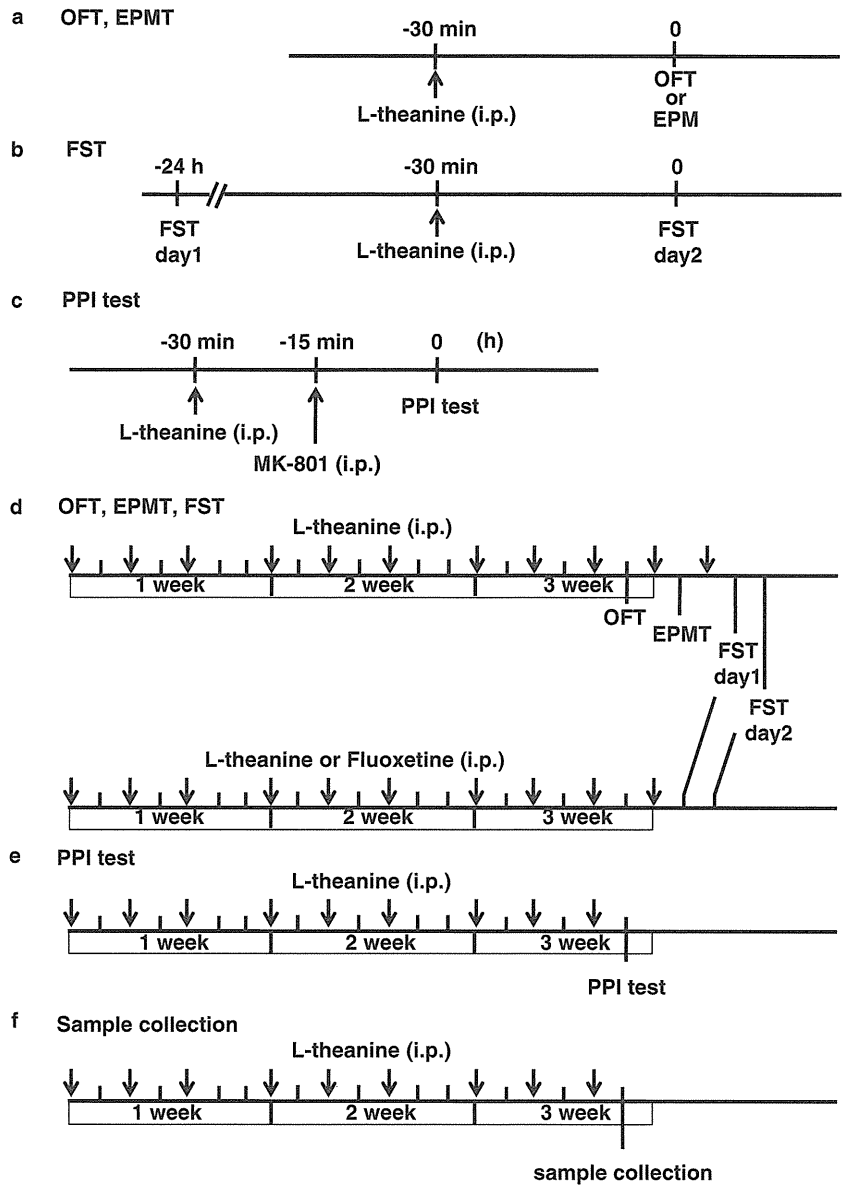

Fig. 1 Schematic representation of experimental schedules. The schedule for animals receiving **a** open-field test (OFT) or elevated plus-maze test (EPMT), **b** forced swim test (FST), and **c** prepulse inhibition (PPI) test by single administration. The schedule for animals receiving **d** OFT, EPMT, FST, and **e** PPI test after subchronic administration. **f** The schedule for sampling brain for western blot analysis. *i. p.* intraperitoneal administration

were dissolved in saline and administered intraperitoneally (i.p.) in a volume of 0.1 ml/10 g body weight.

Single administration of L-theanine

In the OFT, EPMT, and FST, saline or L-theanine (4, 20, 100 mg/kg) was administered intraperitoneally (i.p.) 30 min before the test. In the PPI test, the drugs were administered i.p. 30 min (saline or L-theanine; 1 or 10 mg/kg) and 15 min (MK-801; 0.5 mg/kg) prior to the test, respectively. These doses were chosen because administration of 1 mg/kg i.p. was reported to produce pharmacological effects of L-theanine such as prevention of memory impairment induced by repeated cerebral ischemia in rats (Egashira et al. 2008) and neuroprotective effect on focal cerebral ischemia in mice (Egashira et al. 2007). We examined whether L-theanine has effects on behaviors assessed with EPMT or FST at doses more than 1 mg/kg in order to avoid false negative results because of inadequate dose.

Subchronic administration of L-theanine

In the subchronic administration, L-theanine (0.4, 2, 10 mg/kg), fluoxetine (10 mg/kg), or saline was administered i. p. to 5-weeks old mice. We performed administration three times in every other day in a week for 3 weeks. Behavioral tests were carried out 24 h after the last administration or later.

Behavioral test

Open-field test

The spontaneous locomotor activity was quantified in an open-field (OF-2400R: O'Hara & Co., Ltd., Tokyo, Japan) which consisted of a white plastic box (50 cm [length]× 50 cm [width]×40 cm [height]) illuminated by a 30-lux light. The number of line-crossings, rearing, and climbing were scored for 15 min. A line-crossing was counted when all four paws were moved from one square (10×10 cm) to another. Line-crossings were classified into "central" and "peripheral" based on the position of the square entered. We considered "center part" as the central 30×30 cm square. Animals' movement was monitored by a charge coupled device camera for 15 min. The distance travelled were measured and analyzed automatically on a Macintosh computer using the Image OFCR (O'Hara & Co., ltd., Tokyo, Japan), a modified software based on the public-domain NIH Image Program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Elevated plus-maze test

Mice were placed in the center of an elevated plus-maze (located 50 cm above the floor; arms of 25 cm [length]× 5 cm [width] with 15-cm tall walls on the closed arms), and their behavior was recorded for 10 min. The time spent on the closed and open arms, as well as the number of entry into open and closed arms and crossing distances, were measured and analyzed by a video-tracking software, Image EPM (O'Hara & Co., ltd., Tokyo, Japan). The time spent on the open arms was considered to be correlated with anxiety-like behavior (Lister 1987).

Forced swim test

The forced swim procedure used was similar to that of Porsolt et al. (1977). Two swim sessions were conducted between 10:00 and 14:00 in two consecutive days. In the first session (pre-test), mice were placed individually for 6 min into a plastic cylinder (15 cm diameter×25 cm height) containing 15 cm height from the bottom of water at 24±0.5°C. The second session was followed 24 h later. All test sessions were recorded by a video camera positioned on the side of the cylinders. The videotapes were analyzed and scored by an observer who was blind to the drug administration status. Immobility, swimming, and climbing times were measured. Climbing behavior consisted of upward-directed movements of the forepaws along the side of the swim chamber. Swimming behavior was defined as movement (usually horizontal) throughout the swim chamber, which usually included crossing into another quadrant. Immobility was assigned when no additional activity was observed other than that required to keeping the mice's head above water.

Prepulse inhibition

Acoustic startle and PPI responses were measured in a startle chamber (O'Hara & Co., Ltd., Tokyo, Japan) adapted for mice. The chosen paradigm was modified from Braff and Geyer (1990). Briefly, we used to assess startle amplitude, habituation, and PPI response with acoustic stimuli of 120 dB, a single prepulse interval (100 ms), and four different prepulse intensities [8, 12, 16 and 20 dB above background noise (white noise of 70 dB)]. Each mouse was placed in the startle chamber and initially acclimatized for 3 min with the background noise alone. Mouse was then subjected to 30 startle trials, each trial consisting of one of five conditions: (1) a 40 ms, 120 dB noise burst presented alone; (2–5) a 40 ms, 120 dB noise burst after prepulses by 100 ms (20 ms noise burst) that were 8, 12, 16, and 20 dB above background noise (i.e., 78, 82, 86, or 90 dB prepulse, respectively); or (6) no stimulus (background noise alone), which was used to measure baseline movement in the chamber. In PPI tests, these five trial types (2–6) were each repeated four times in a random order to give 20 trials. Trial type (1) was consecutively done as the first five trials and the last five trials. The intertrial interval was randomly chosen between 15 and 30 s. Analysis of PPI was based on the mean of the four trials for each trial type. The percentage PPI of a startle response was calculated as follows: PPI=100−[(prepulse and pulse stimulus trials−average of 10 no stimulus trials)×100/(pulse-alone trials−average of 10 no stimulus trials)]

Western blotting

The separated hippocampus and cerebral cortex were homogenized in lysis buffer and the protein concentration in samples was determined before the western blotting analysis as previously described (Numakawa et al. 2009, 2003). The equivalent amounts of total protein were assayed for each immunoblotting. Primary antibodies were used at the following dilutions: anti-TrkB (1:1,000, BD Biosciences, San Jose, CA), anti-GluR1 (1:1,000, CHEMICON, CA), anti-p75 (1:1,000, Promega Inc., WI), anti-NR2B (1:500, SIGMA, MO), and anti-BDNF (1:200, Santa Cruz Biotechnology Inc., Santa Cruz, CA) antibodies. The changes in protein expression are indicated as a ratio that was normalized to a control in each experiment. The immunoreactivity was quantified by using Lane & Spot Analyzer software (ATTO Corporation, Tokyo).

Intracellular Ca^{2+} imaging in cultured cortical neurons

Ca^{2+} imaging was performed using Fluo-3 AM dye (Molecular Probes, Inc., WI) as previously reported (Numakawa et al. 2004, 2002). The changes in the Fluo-3 intensity through the fluorescent microscope were analyzed and quantified using Slide Book TM 3.0 (Intelligent Imaging Innovations Inc., CO) from randomly selected cell bodies in cortical neuronal cultures prepared from postnatal day 1- or 2-day-old rats (Wistar, SLC, Shizuoka) as described previously (Numakawa et al. 2002). NMDA antagonist MK-801 (final 10 μM) or AP-5 (final 10 μM) was added to the neurons by bath application at 5–6 days in vitro for 30 min and then L-theanine (final 1 μM) was added. All imaging experiments were performed at least three times.

Statistical analysis

A one-way factorial analysis of variance (ANOVA) or repeated measures analysis of variance (RMANOVA) was used to assess each behavior. We used the Dunnett's test to compare the saline group to L-theanine-treated groups in the FST and EPMT. Behavioral experimental data are presented as mean±standard error (SE) and western blotting and intracellular Ca^{2+} influx analysis data are presented as mean±standard deviation (SD). Percentage of PPI was analyzed by RMANOVA and followed by Bonferroni-corrected pairwise comparisons as the post hoc tests. A p value <0.05 was regarded as statistically significant. Analyses were conducted with SPSS ver. 11.0 (SPSS Japan Inc., Tokyo, Japan).

Results

Effects of single administration of L-theanine on behaviors

In the OFT, L-theanine did not have a significant effect on the distance traveled (i.e., spontaneous motor activity) (Supplementary Figure S1). In the EPMT and FST, L-theanine did not exert any significant effect (Supplementary Figures S2 and S3). However, when we examined the effect of L-theanine on MK-801-induced deficits in the PPI test, pretreatment with L-theanine significantly improved PPI (Fig. 2a) (prepulse, F [3, 300]=18.5, p=0.0001 [Huynh–Feldt correction]; L-theanine×MK-801 interaction: F [2, 100]=4.5, p=0.013). MK-801-induced augmentation of startle intensity was not significantly affected by pretreatment with L-theanine (Fig. 2b). Single administration of L-theanine did not have any significant effect on the baseline PPI (i.e., PPI without MK-801 pretreatment) or startle intensity (Fig. 2a, b).

Effect of subchronic administration of L-theanine on behaviors

Although subchronic administration of L-theanine did not have a significant effect on the OFT or EPMT results (Supplementary Figures S4 and S5), significantly reduced immobilization time in the FST had nearly the same effect as fluoxetine (F=4.10, df=3, 38, p=0.014 [Fig. 3b]). Reduced immobilization time was caused by increase in swimming time (F=3.64, df=3, 38, p=0.022 [Fig. 3a]). Climbing activity tended to increase by fluoxetine (F=2.86, df=3, 38, p=0.051 [Fig. 3c]). This anti-depression-like behavior was reproducible as described in supplementary Fig. S6. Furthermore, subchronic administration of L-theanine significantly improved the baseline PPI (prepulse×L-theanine interaction, F [8.77, 213]=2.6, p=0.009 [Huynh–Feldt correction]) (Fig. 4a), especially at the dose of 2 mg/kg (F [3, 73]= 4.21, p=0.008). The acoustic startle intensity was not significantly altered by L-theanine (Fig. 4b).

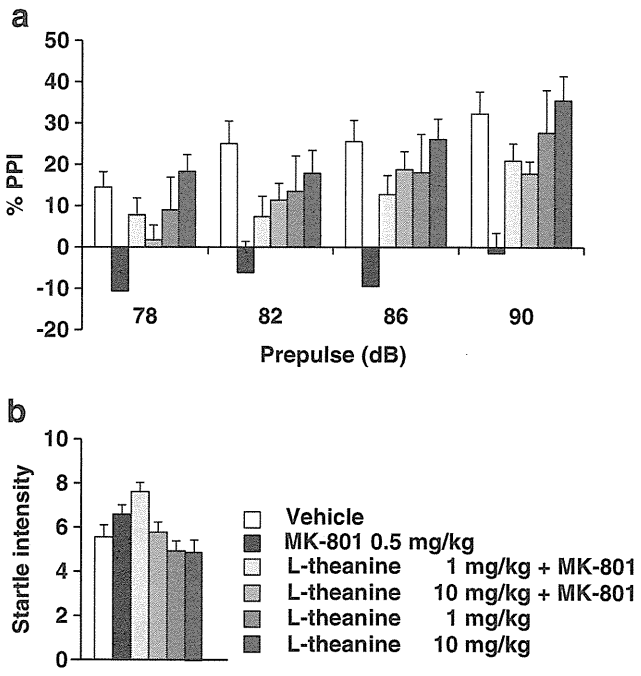

Fig. 2 Effects of single administration of L-theanine on MK-801-induced disruption of prepulse inhibition (PPI) test of acoustic startle. L-theanine (1, 10 mg/kg) or vehicle was injected 30 min and MK-801 (0.5 mg/kg) or vehicle was 15 min before the test. **a** PPI of vehicle-administered control and L-theanine-administered mice with 78, 82, 86, and 90 dB prepulse stimuli were measured with or without MK-801 treatment. **b** Acoustic startle intensity of vehicle-treated control and L-theanine-treated mice was measured with or without MK-801 treatment. The results are presented as means±SEM (n=15–20/group). [prepulse; F (3, 300)=18.5, p=0.0001 (Huynh–Feldt correction); L-theanine×MK-801 interaction; F (2, 100)=4.54, p=0.013; p= 0.01 (MK-801 vs. L-theanine1 mg/kg pretreat+MK-801), p=0.005 (MK-801 vs. L-theanine 10 mg/kg pretreat).]

Biochemical analysis of the effect of L-theanine

As shown in Fig. 5a, BDNF levels significantly increased in the hippocampus after subchronic administration of L-theanine (F [3, 12]=9.8, p=0.0015). In contrast, TrkB, p75 (a low-affinity receptor for BDNF), NMDA (NR2B), and AMPA (GluR1) receptor subunits were not significantly altered (Fig. 5b–e). In samples obtained from the cortex, BDNF levels were not significantly altered (F [3, 12]=0.44, p=0.73; Supplementary Figure S7a). The cortical levels of TrkB, p75, NR2B, and GluR1 were also not significantly altered (Supplementary Figure S7b–e).

To clarify whether L-theanine acts on NMDA receptors as an agonist or antagonist, the Ca^{2+} influx were examined by using Fluo-3 as an indicator. In several studies, including our previous one, cultured neurons are stimulated by glutamate at the concentration of 1 μM (DeCoster et al. 2002; Numakawa et al. 2002). Thus, we examined the effect of 1 μM of L-theanine on the Ca^{2+} influx, as we speculated its glutamate-like action. As shown in Fig. 6a, b, L-theanine significantly increased the Ca^{2+} influx in cultured cortical neurons. Furthermore, both MK-801 (a non-competitive NMDA receptor antagonist) and AP-5 (a competitive NMDA receptor antagonist) suppressed the intracellular Ca^{2+} influx induced by L-theanine (Fig. 6c, d).

Discussion

We found that single administration of L-theanine significantly attenuated the MK-801-induced deficits in PPI (Fig. 2a), although the effect on the baseline PPI was not significant (i.e., PPI without MK-801 pretreatment). In contrast, subchronic treatment with L-theanine significantly improved the baseline PPI (Fig. 4a). To our knowledge, this study is the first to provide evidence for the effects of L-theanine on sensorimotor gating. Impaired sensorimotor gating as indicated by decreased PPI is a feature of patients with schizophrenia (Braff et al. 2001; Kunugi et al. 2007). Moreover, antipsychotic drugs suppress the disruption of PPI induced by NMDA receptor antagonists such as MK-801 and phencyclidine (Bubenikova et al. 2005; Fejgin et al. 2007; Ishii et al. 2010; Wiley and Kennedy 2002). Therefore, our results suggest that L-theanine exerts an antipsychotic-like effect. Of course, we cannot deny the possibility that another receptors such as AMPA and kinate receptors relate to the effect of L-theanine on PPI. Further detailed experiences are required to address this issue.

Interestingly, we also found that subchronic treatment with L-theanine elevated the swimming time and reduced the immobilization time in the FST (Fig. 3a, b) but did not increase spontaneous locomotor activity in the OFT (Supplementary Figure S4). Immobilization in the FST has been considered as a despair-like behavior, which can be decreased by administration of antidepressants (Petit-Demouliere et al. 2005; Porsolt et al. 1977). It is therefore possible that L-theanine exerts an antidepressant-like effect, which is in accordance with Yin et al. (2011). In our result, although L-theanine has an antidepressant-like activity comparable to that of fluoxetine in immobilization time (Fig. 3b), swimming time is clearly different and excellent in high dose of L-theanine-treated group (Fig. 3a). In addition, immobility in the FST is also reduced by some atypical antipsychotic drugs such as olanzapine and clozapine (Noda et al. 1995; Weiner et al. 2003), which may have therapeutic effects on the negative symptoms of schizophrenia. Together with the PPI findings, the results of the FST indicate that L-theanine exerts antipsychotic-like effects similar to the effects of some atypical antipsychotic drugs, although the mechanism of action is clearly different.

We did not find any notable changes in the locomotor activity in the OFT or EPMT after single or subchronic administration of L-theanine. Anxiety-like behavior was not influenced by either single or subchronic treatment with L-theanine, when assessed by the time spent in the center in the OFT, time spent in the open arms in the EPMT, or startle intensity in the PPI test. Heese et al. (2009) reported that L-theanine alone did not exert any anxiolytic-like effect in the

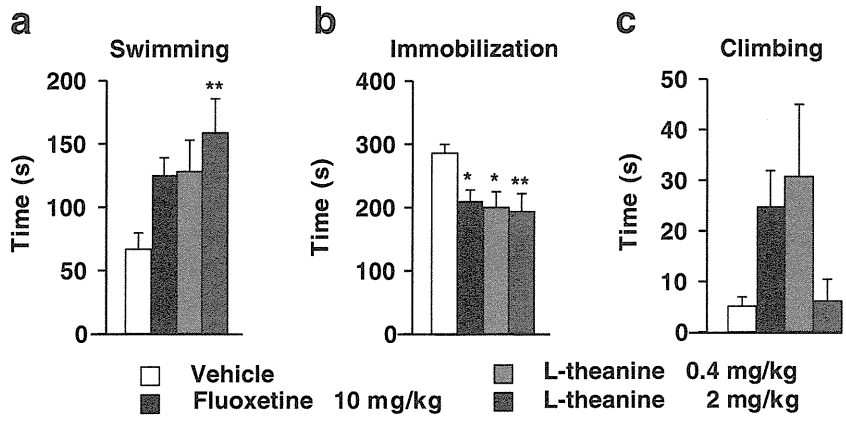

Fig. 3 Effects of subchronic administration of L-theanine on forced swimming test (FST). L-theanine (0.4, 2 mg/kg, i.p.) or fluoxetine (10 mg/kg) or vehicle was subchronically administered every other day for 3 weeks. **a** Swimming time, **b** immobilization time, and **c** climbing time were analyzed. The results are presented as means±SEM (n=10/group). *p<0.05; **p<0.01 when compared with vehicle control (Dunnett's test)

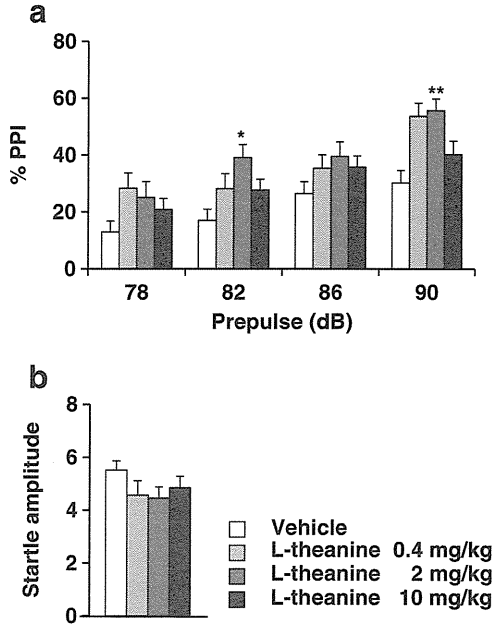

Fig. 4 Effects of subchronic administration of L-theanine on the baseline prepulse inhibition (PPI) test (i.e., without pretreatment of MK-801). L-theanine (0.4, 2, 10 mg/kg, i.p.) or vehicle was subchronically administered every other day for 3 weeks. **a** Percent PPI and **b** startle intensity. The results are presented as mean±SEM (n=17–21/group). [prepulse×L-theanine interaction; F (8.77,213)=2.56, p=0.009 (Huynh–Feldt correction) by RMANOVA analysis. F (3,73)=4.21, p=0.008 (2 mg/kg administration). *p<0.005; **p<0.001; Bonferroni-corrected pairwise comparisons as the post hoc tests.]

EPMT; however, a synergistic effect was observed when the combined treatment with a benzodiazepine drug, midazolam, was administered. In addition, a previous human study showed that the relaxing effect of L-theanine was only found in the baseline condition and not in the state of induced anxiety (Lu et al. 2004). But recent clinical study shows that L-theanine relieves positive, activation, and anxiety symptoms in patients with schizophrenia and schizoaffective disorder (Ritsner et al. 2011). In addition, a combination of green tea extract and L-theanine improves memory and attention in subjects with mild cognitive impairment in human study (Park et al. 2011). Together, these findings and ours indicate that L-theanine might be effective under the specific state of anxiety or improve cognitive deficits characteristic to schizophrenic patients.

It is interesting to note the difference in behavioral effects between single and subchronic administrations of L-theanine. Single administration of L-theanine did not affect behaviors compared with saline-treated mice in the FST or baseline PPI (Supplementary Figure S3 and Fig. 2, respectively). In contrast, clear behavioral effects were observed in the FST (Fig. 3) and baseline PPI (Fig. 4) after subchronic administration of L-theanine. It can be postulated that the reduced despair-like behavior in the FST and the improvement of baseline PPI resulted from plastic changes in the brain via increase in the BDNF levels, during subchronic (3-week duration) administration of L-theanine.

Many researchers have suggested that the mechanism of action of antidepressants involves increased BDNF expression in the hippocampus. This may explain why the clinical effects of antidepressants are evident only after a few weeks of administration of these drugs (Castren and Rantamaki

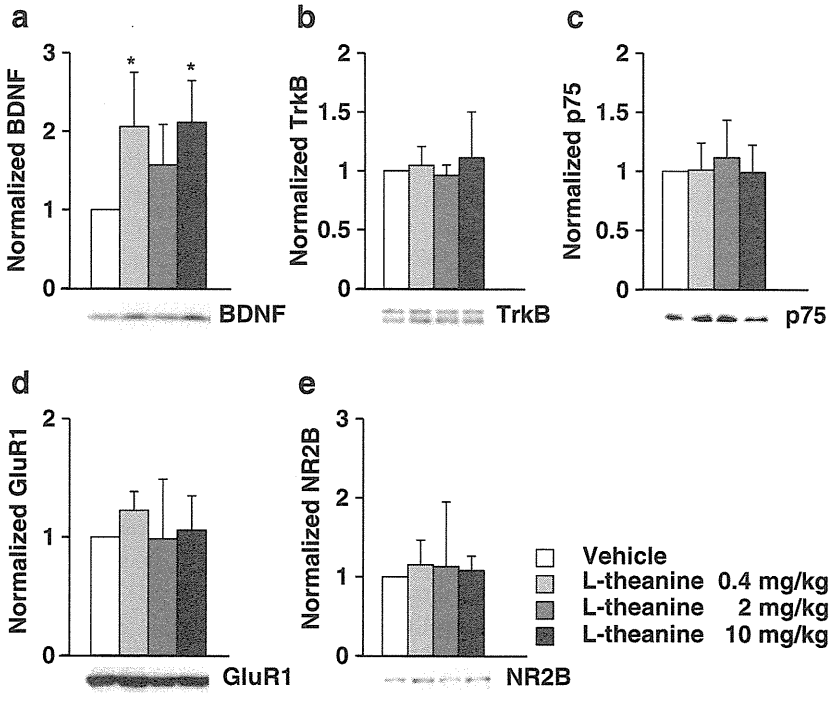

Fig. 5 Subchronic administration of L-theanine increased BDNF expression in the hippocampus. Vehicle or L-theanine (0.4, 2, 10 mg/kg) was subchronically administered (i.p.) and hippocampus was removed. The equivalent amounts of total protein were applied to western blot analysis. Representative images of immunoblotting with anti-BDNF (**a**), anti-TrkB (**b**), anti-p75(**c**), anti-GluR1 (**d**), and anti-NR2A (**e**) antibodies were shown. The levels of BDNF, TrkB, p75, GluR1, and NR2A were quantified by densitometry, respectively. Data represent mean±S.D. (n=4, the number of animals for each experimental dose). Data were normalized to a level of vehicle control (without L-theanine). *p<0.01 when compared with vehicle control (Dunnett's test)

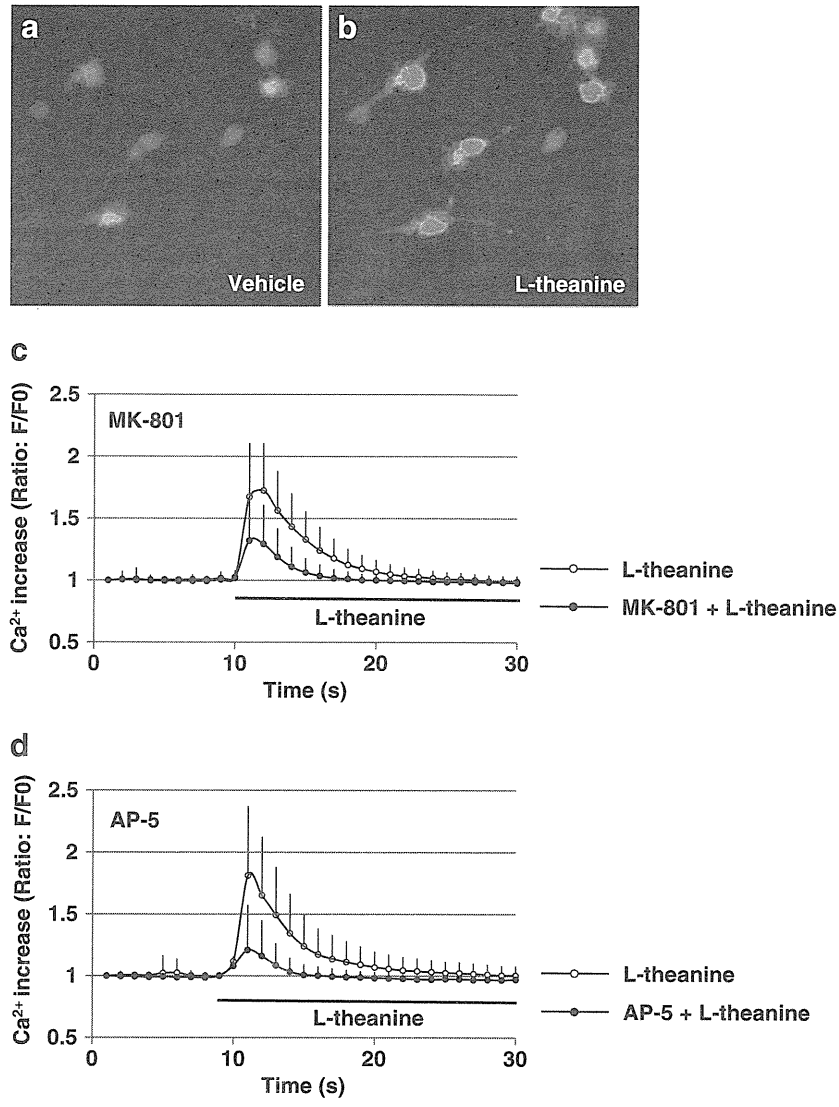

Fig. 6 Intracellular Ca^{2+} concentration was increased by L-theanine and was partially attenuated by NMDA receptor antagonist MK-801 and AP-5 in cultured neurons. **a** For the imaging of Ca^{2+}, Fluo-3 dye was loaded cortical neurons. **a** Before and **b** 4 s after L-theanine (1 μM) addition, photo imaging was taken. **c**, **d** Plots summarize data from **c** 171 and 188 (with or without 10 μM MK-801, respectively) and **d** 51 and 50 (with or without 10 μM AP-5, respectively) selected cells from sister cultures at 6 days in vitro. The ratio (F/F_0) of fluorescence was calculated from the intensities of fluorescence before and after L-theanine application (F/F_0; stimulated level/basal level). Data represent mean±SD

2010; Duman and Monteggia 2006; Martinowich et al. 2007). BDNF has also been implicated in schizophrenia (Durany et al. 2001; Green et al. 2010) and in the mechanism of action of antipsychotic drugs (Pillai 2008). We showed, for the first time, that the endogenous BDNF level in the hippocampus increased after subchronic L-theanine treatment, although the levels of TrkB, p75, and other glutamate receptor-related molecules GluR1 and NR2B were unaltered (Fig. 5). We therefore suggest that L-theanine exerts its effects on behavior at least in part through upregulation of BDNF. Plastic changes in the hippocampus via increased BDNF expression may be involved in the improvement of the baseline PPI by subchronic treatment with L-theanine, because the hippocampus is one of the regulatory regions of PPI (Swerdlow et al. 2001) as well as FST (Santarelli et al. 2003). It is noteworthy that Yokogoshi et al. (1998a) reported that L-theanine significantly increased serotonin levels in the striatum, hippocampus, and hypothalamus. Furthermore, chronic antidepressant administration induces BDNF expression in the brain (Nibuya et al. 1995; Santarelli et al. 2003). Given these findings, it is possible that the induction of BDNF expression might be caused, at least in part, by serotonin induced by subchronic administration of L-theanine.

It is well known that BDNF levels are regulated via glutamate-mediated neuronal activity, especially via the functions of NMDA receptors (Castren et al. 1993; Mattson 2008; Patterson et al. 1992; Zafra et al. 1991). It is therefore feasible to postulate that subchronic administration of L-theanine upregulates BDNF via its effects on NMDA receptors. An increase in intracellular Ca^{2+} concentration is involved in the activation of calmodulin kinase II (CaMKII), with a resultant increase in BDNF expression (Takeuchi et al. 2000). Furthermore, NMDA receptor-mediated Ca^{2+} influx is thought to regulate BDNF levels

(Numakawa et al. 2010; Shieh et al. 1998; Tao et al. 1998). To clarify this possibility, we examined whether L-theanine directly alters intracellular Ca^{2+} in cultured cortical neurons. Ca^{2+} imaging analysis showed that acute and transient Ca^{2+} increase was triggered by L-theanine (Fig. 6). This L-theanine-induced increase in Ca^{2+} level was suppressed in the presence of non-competitive and competitive NMDA receptor antagonists (MK-801 and AP-5, respectively) (Fig. 6c, d), suggesting that L-theanine exerts its effects, at least in part, through agonistic action on NMDA receptors.

The NMDA receptor hypofunction hypothesis of schizophrenia suggests that increase in the NMDA receptor function via pharmacological manipulation could provide a potential new strategy for the management of schizophrenia (Goff and Coyle 2001; Javitt and Zukin 1991). Currently, the glycine modulatory site on the NMDA receptor is one of the most attractive therapeutic targets for the treatment of patients with schizophrenia (Lipina et al. 2005). Many studies have shown that D-amino acids such as D-serine as well as glycine bind to the NMDA receptor as co-agonists of glutamate, and thereby attenuate deficits in PPI induced by the NMDA receptor antagonist MK-801 (Kanahara et al. 2008). Our results suggest that L-theanine exerts antipsychotic-like effects, and also has an agonistic action on the NMDA receptor. However, L-theanine has also been shown to bind to other glutamate receptors, such as AMPA and kainate receptors (Kakuda et al. 2002). Further studies to examine the detailed downstream signaling of L-theanine are required.

Acknowledgments This study was supported by the Health and Labor Sciences Research Grants (Comprehensive Research on Disability, Health, and Welfare), Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS), CREST, JST, and "Understanding of molecular and environmental bases for brain health" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Financial disclosures All authors repot no biomedical financial interests or potential conflicts of interest.

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Psychiatry and Clinical Neurosciences 2011; 65: 280–285 doi:10.1111/j.1440-1819.2011.02193.x

Regular Article

Nominal association between a polymorphism in *DGKH* and bipolar disorder detected in a meta-analysis of East Asian case–control samples

Atsushi Takata, MD,[1,3] Hiroaki Kawasaki, MD, PhD,[1]* Yoshimi Iwayama, MS,[2] Kazuo Yamada, MD, PhD,[2] Leo Gotoh, PhD,[1] Hiroshi Mitsuyasu, MD, PhD,[1] Tomofumi Miura, MD, PhD,[1] Tadafumi Kato, MD, PhD,[3] Takeo Yoshikawa, MD, PhD[1] and Shigenobu Kanba, MD, PhD[1]

[1]*Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, and Laboratories for* [2]*Molecular Psychiatry and* [3]*Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Saitama, Japan*

Aim: Recent genome-wide association studies (GWAS) of bipolar disorder (BD) have detected new candidate genes, including *DGKH*, *DFNB31* and *SORCS2*. However, the results of these GWAS were not necessarily consistent, indicating the importance of replication studies. In this study, we tested the genetic association of *DGKH*, *DFNB31* and *SORCS2* with BD.

Methods: We genotyped 18 single-nucleotide polymorphisms (SNP) in *DGKH*, *DFNB31* and *SORCS2* using Japanese samples (366 cases and 370 controls). We also performed a meta-analysis of four SNP in *DGKH*, using the previously published allele frequency data of Han-Chinese case–control samples (1139 cases and 1138 controls).

Results: In the association analysis using Japanese samples, a SNP in *SORCS2* (rs10937823) showed nominal genotypic association. However, we could not find any association in an additional analysis of tag SNP around rs10937823. In the meta-analysis of SNP in *DGKH*, rs9315897, which was not significantly associated with BD in the previous Chinese study, showed nominal association.

Conclusion: Although the association was not strong, the result of this study would support the association between *DGKH* and BD.

Key words: DFNB31, genome-wide association studies, manic–depressive illness, mood disorder, SORCS2.

FAMILY, TWIN AND adoption studies have consistently demonstrated the contribution of inherited genetic variation on risk for bipolar disorder (BD).[1] Therefore, numerous genetic studies, including linkage mapping and candidate gene studies, have been carried out. However, the results of these studies have largely been inconsistent. After the era of linkage study and candidate gene approach, genome-wide association studies (GWAS), which investigate 500 000 to 1 000 000 single-nucleotide polymorphisms (SNP) throughout the genome using DNA microarray, have become popular. For BD, an increasing number of GWAS, including meta-analyses, have been conducted.[2–7,16,17] Meta-analyses of GWAS data of BD and major depressive disorder were also performed.[8,9] These studies identified many previously unsuspected candidate genes.

DGKH, *DFNB31* and *SORCS2* are included in these new candidate genes for BD, as well as other promising genes, such as *ANK3*, *CACNA1C*,[3] *PBRM1*[8] and so on. The association of *DGKH*, *DFNB31* and

Correspondence: Hiroaki Kawasaki, MD, PhD, Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Email: hiroakik@npsych.med.kyushu-u.ac.jp
Received 12 August 2010; revised 15 December 2010; accepted 12 January 2011.

SORCS2 with BD was identified in the first reported GWAS using a DNA pooling strategy followed by individual genotyping.[2] All of these genes are expressed in the brain and have some functional implication with neuropsychiatric disorders. Diacylglycerol kinase (DGK) eta, encoded by *DGKH*, is a member of the DGK family that plays an important role in the inositol pathway, the putative site of action of lithium.[10] *DFNB31* encodes whirlin, which is also known as Cip98. This protein interacts with a calmodulin-dependent serine kinase and is suggested to be involved in the formation of scaffolding protein complex and in synaptic transmission.[11] A mutation in this gene is known to cause Usher syndrome,[12] which is characterized by hearing impairment and progressive vision loss. One study reported frequent comorbidity of bipolar disorder or depressive disorder in Usher syndrome patients.[13] *SORCS2*, encoding a VSP10 domain containing-receptor, is expressed in both the developing and adult brain.[14] While the ligand for SORCS2 is so far unknown, highly homologous other members of the SORCS VSP10 domain-containing-receptor family, SORCS1 and SORCS3, are known to bind several growth factors, such as NGF and PDGF.[15] These findings further make *DGKH*, *DFNB31* and *SORCS2* good candidates for BD. However, GWAS usually investigate hundreds of thousands SNP and always involve the potential for false positive results. In fact, some inconsistent results about these genes were reported in other GWAS for BD.[3–7,16,17]

Several replication studies of *DGKH*, *DFNB31* and *SORCS2* were also reported and they include both positive and negative results. A study in Sardinian samples (197 cases and 300 controls) detected a haplotypic association between BD and *DGKH*.[18] Another study that examined 36 tag SNP from *DGKH* in a Scandinavian population (594 cases and 1421 controls) reported no association.[19] A replication study of GWAS that investigated 26 SNP, including those from *DGKH*, *DFNB31* and *SORCS2* using a Finnish family cohort (723 individuals from 180 families), reported associations of *DFNB31* and *SORCS2* but no association of *DGKH* with BD.[20] Recently, Zeng *et al.* reported a strong haplotypic association (minimum $P = 3.87 \times 10^{-6}$) between *DGKH* and BD in a Han-Chinese case–control cohort.[21]

In this study, we investigated associations of *DGKH*, *DFNB31* and *SORCS2* with BD using Japanese case–control samples (366 cases and 370 controls). Furthermore, we conducted a meta-analysis of four SNP in *DGKH*, which are overlapped with the SNP investigated by Zeng *et al.* In total, 1505 cases and 1508 control samples were used for the meta-analysis.

METHODS

The Japanese case–control samples consisted of 366 patients with bipolar I disorder (BDI), bipolar II disorder (BDII) or schizoaffective disorder bipolar type (SAB) (257 BDI, 104 BDII and five SAB; 181 men and 185 women, aged 50.1 ± 13.4 years) and 370 control subjects (185 men and 185 women aged 50.6 ± 12.6 years). All subjects were unrelated and ethnically Japanese. The patients were diagnosed by at least two experienced psychiatrists according to the DSM-IV criteria on the basis of unstructured interviews and reviews of their medical records. All healthy control subjects were also psychiatrically screened on the basis of unstructured interviews. The objective of the present study was clearly explained, and written informed consent was obtained from all the participants. The characteristics of the Han-Chinese cohort used for meta-analysis were described elsewhere.[21] This study was approved by the ethics committees of Kyushu University Graduate School of Medicine and RIKEN Brain Science Institute.

For SNP selection, we focused on the SNP that were reported in a previous GWAS by Baum *et al.* and that cause non-synonymous amino acid substitutions and possibly affect the function of encoded proteins. SNP chosen from Baum *et al.* included six SNP whose associations were detected in the follow-up individual genotyping (three in *DGKH*, one in *DFNB31* and two in *SORCS2*, reported *P*-values ranging from 0.0005 to 1.5×10^{-8}). Three SNP in *DGKH* indicated to be associated in both of the two independent pooling sample sets were additionally selected. The selected non-synonymous SNP consisted of two SNP in *DGKH*, five SNP in *DFNB31* and two SNP in *SORCS2*. The total number of selected SNP was eighteen. Genotyping was performed using TaqMan assay (Applied Biosystems, Foster City, CA, USA). Differences in allele and genotype frequencies between BD and controls were evaluated with Fisher's exact test. Deviations from Hardy–Weinberg equilibrium (HWE), structures of linkage disequilibrium (LD) blocks and haplotypic associations were analyzed using Haploview version 4.1 software,[22] (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/