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A Combination of Green Tea Extract and L-Theanine Improves Memory and Attention in Subjects with Mild Cognitive Impairment: A Double-Blind Placebo-Controlled Study

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ABSTRACT A combination of green tea extract and L-theanine (LGNC-07) has been reported to have beneficial effects on cognition in animal studies. In this randomized, double-blind, placebo-controlled study, the effect of LGNC-07 on memory and attention in subjects with mild cognitive impairment (MCI) was investigated. Ninety-one MCI subjects whose Mini Mental State Examination-K (MMSE-K) scores were between 21 and 26 and who were in either stage 2 or 3 on the Global Deterioration Scale were enrolled in this study. The treatment group (13 men, 32 women; 57.58 ± 9.45 years) took 1,680 mg of LGNC-07, and the placebo group (12 men, 34 women; 56.28 ± 9.92 years) received an equivalent amount of maltodextrin and lactose for 16 weeks. Neuropsychological tests (Rey–Kim memory test and Stroop color–word test) and electroencephalography were conducted to evaluate the effect of LGNC-07 on memory and attention. Further analyses were stratified by baseline severity to evaluate treatment response on the degree of impairment (MMSE-K 21–23 and 24–26). LGNC-07 led to improvements in memory by marginally increasing delayed recognition in the Rey–Kim memory test ($P = .0572$). Stratified analyses showed that LGNC-07 improved memory and selective attention by significantly increasing the Rey–Kim memory quotient and word reading in the subjects with MMSE-K scores of 21–23 (LGNC-07, $n = 11$; placebo, $n = 9$). Electroencephalograms were recorded in 24 randomly selected subjects hourly for 3 hours in eye-open, eye-closed, and reading states after a single dose of LGNC-07 (LGNC-07, $n = 12$; placebo, $n = 12$). Brain theta waves, an indicator of cognitive alertness, were increased significantly in the temporal, frontal, parietal, and occipital areas after 3 hours in the eye-open and reading states. Therefore, this study suggests that LGNC-07 has potential as an intervention for cognitive improvement.

KEY WORDS: • *attention* • *electroencephalography* • *green tea* • *memory* • *neuropsychological test* • *L-theanine*

INTRODUCTION

COGNITION-RELATED MENTAL PROBLEMS as a social issue in modern society are growing in importance because they are often associated with individual survival, professional life, and the economy. Reports about subjective memory complaints (SMCs) indicate that the prevalence of these problems in communities is between 20% and 50%, depending on the study.[1,2] Several studies have shown that these problems are also associated with depression, anxiety, and a higher level of education.[3] Although it is still controversial whether SMCs result in future cognitive decline,[4] SMC is acknowledged as an initial criterion of mild cognitive impairment (MCI), a transitional state between cognitive decline due to normal aging and mild Alzheimer's disease (AD).[5] MCI is defined as a deficit in memory performance with normal cognitive function in the absence of a diagnosis of AD.[6] Early diagnosis of MCI subjects shows that they are at high risk of AD conversion. From 10% to 15% of MCI subjects have been reported to progress to AD within a year, whereas only 1–2% of the normal elderly population showed AD conversion.[7] In 2008, the incidence of MCI in those aged over 65 years in Korea was estimated to be 24.08%, or 1,207,836 people (425,593 men and 781,243 women).[8]

Researchers have reported the ameliorating effect of nutraceutical ingredients such as ginkgo extract and soy

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proteins containing isoflavone on cognitive decline;[9,10] however, the outcomes have not been satisfactory. Oral AD therapeutics such as donepezil hydrochloride (Aricept®, Pfizer, New York, NY, USA) do not improve MCI and have undesirable side effects such as nausea, diarrhea, depression, and insomnia, among others.

LGNC-07 is a nutraceutical ingredient that contains green tea extract (GTE) and L-theanine. GTE has been consumed on a daily basis in many Asian countries, and its neuroprotective effects have been studied by many researchers. Lee *et al.*[11] reported that GTE attenuated neuroblastoma cell death induced by amyloid β (Aβ), which is regulated through nuclear factor κB, extracellular signal-regulated kinase, and p38 mitogen-activated protein kinase pathways. Anticholinesterase activity in both human and animal erythrocytes suggests the potential retardation of the progression of cognitive impairment.[12,13] L-Theanine is well known for its relaxing effect on mental stress,[14,15] and further research has demonstrated its memory improvement ability in a scopolamine-induced animal study.[16] In our previous studies, LGNC-07 led to an improvement in memory and learning in cognition impairment animal models. Scopolamine-induced memory impairment was ameliorated by inhibiting acetylcholinesterase activity,[17] and protection against cell death and secretase inactivation were shown to contribute to memory improvement in the $A\beta_{1-42}$ animal model.[18]

Cognitive changes in healthy or elderly subjects by memory-enhancing agents have been assessed by electroencephalography (EEG) as well as by neuropsychological tests.[19–25] It was reported that the theta power spectrum was related to active mental performance such as learning and goal-driven tasks.[24,25] However, there have been no studies about the effect of LGNC-07 on cognitive function using neuropsychological testing and EEG in human subjects.

In this study, a placebo-controlled double-blind clinical study was conducted to investigate whether LGNC-07 can improve cognition using the Rey–Kim memory test, Stroop color–word reading test, and EEG on MCI subjects. It was shown that LGNC-07 increased the Rey–Kim memory quotient (MQ) and correct count of Stroop word reading, suggesting potential memory- and attention-enhancing ability of LGNC-07 in subjects with moderate to severe MCI. In addition, LGNC-07 was shown to regulate the activity of the theta power spectrum, which further indicates that it is a promising nutraceutical candidate for the improvement of cognition.

SUBJECTS AND METHODS

Subjects

This study was conducted at Daejeon University Oriental Hospital (Daejeon, Republic of Korea) from January 1, 2008 to March 31, 2009. Ninety-one participants (25 men and 66 women) with SMCs (including MCI) were enrolled in this study. The study protocol was approved by the Daejeon University Oriental Hospital Institutional Review Board. Subjects were voluntarily recruited through institutional advertisements such as internet postings and posters. All subjects signed informed consents after having the study purpose, procedures, randomization, possible risk, discomforts, and guaranteed confidentiality explained. Baseline medical and personal information were gathered at their first visit, including variables such as demographic information, disease history, chest x-ray, and electrocardiogram. Blood and urine laboratory analyses and a basic neuropsychological test (Mini Mental Scale Examination-K [MMSE-K] and Global Deterioration Scale [GDS]) were performed to determine inclusion and exclusion of subjects. Inclusion criteria were as follows: (1) age between 40 and 75 years, (2) Korean literacy, (3) subjective memory complaint, (4) a MMSE-K score of 21–26, and (5) second or third stage of GDS. Subjects were excluded if their symptoms lasted less than a month or they were mentally handicapped, were diagnosed with AD, had other neuropsychological disorders that may have affected cognitive function, abused alcohol/drugs, had liver cirrhosis, had chronic renal failure, were pregnant, were caffeine sensitive or had other serious medical conditions. Food and beverages containing caffeine were restricted during the study period.

Study design

Subjects were assigned to either the placebo or the treatment group by balanced block randomization and instructed to take two 430-mg capsules of treatment or placebo twice a day 30 minutes after meals for 16 weeks. Treatment capsules contained 360 mg of GTE, 60 mg of L-theanine, 5.7 mg of silicone dioxide, and 4.3 mg of magnesium stearate. The placebo contained the same ingredients except for the GTE and L-theanine. The GTE and L-theanine were replaced with 258 mg of maltodextrin and 162 mg of lactose. Concurrent administration of medicine that might affect cognitive function was prohibited, and subjects were put on a 15-day washout period before treatment was initiated. Additional treatments that might affect cognitive function were restricted, and all procedures were conducted by an oriental medicine physician with more than 1 year of experience. Capsules were provided monthly, and any capsules remaining after the study period were counted to evaluate subject compliance.

Neuropsychological tests were performed at baseline and at 8 and 16 weeks after treatment. Adverse events were monitored during the entire study period, and blood analyses were done to evaluate safety.

Neuropsychological tests

MMSE-K. The MMSE-K is the Korean version of the MMSE and is designed to measure various cognitive functions within 10 minutes, with scores ranging from 0 to 30.[26] This instrument is used to assess moderate to severe AD patients and includes cognitive domains such as memory, orientation, language, and attention/concentration.

GDS. The GDS is a test used to assess clinical symptoms and severity of AD (or dementia). It consists of a

description of seven major, clinically distinguishable dementia stages, ranging from ''no cognitive decline'' (GDS 1) to ''very severe'' (GDS 4–7), with ''normal with subjective memory impairment'' (GDS 2) and ''mild dementia'' (GDS 3) as the intermediate stages.[27]

Rey–Kim memory test. The Rey–Kim memory test is a standardized Korean version of the Rey Auditory Verbal Learning Test.[28,29] It contains a Verbal Learning Test (K-AVLT) for verbal memory and a Complex Figure Test (K-CFT) for visuospatial memory. The K-AVLT includes five successive presentations of 15 words followed by a free recall, a 20-minute delayed recall, and a 20-minute delayed recognition trial. For the K-CFT test, copy, immediate recall, and delayed recall were performed 0 and 20 minutes after drawing a diagram from the Rey Complex Figure. The MQ (which is the sum of standardized conversion scores of Trials 1–5 [K-A: trials 1–5]), K-A (delayed recall), K-A (delayed recognition), K-C (immediate recall), K-C (delayed recall), and standardized conversion scores for each test were used for statistical analysis.

Stroop color–word reading test. The Stroop color–word reading test administered in this study is a subgroup test of the Samsung Neuropsychological Screening Battery.[30] It is used to test selective attention to reflect prefrontal cortex activation. Subjects were given stimuli consisting of color words written in the same color as the words themselves (*e.g.*, the word ''red'' in red letters) and color words that were written in a color different from the words (*e.g.*, the word ''red'' in green letters). The subjects were guided to suppress cognitive interference and habitual response during the test, and their cognitive flexibility was evaluated. The number of correct words and color readings was counted to evaluate subject attention.

EEG

Twenty-four subjects were randomly selected for EEG measurement from the initial study group. The subjects were placed in a separate quiet room in a comfortable bed, and baseline recordings were conducted for 15 minutes: 5 minutes with eyes-open, 5 minutes with eyes-closed, and 5 minutes while reading short stories. The subjects took four capsules of treatment or placebo right after the baseline measurement, and the rest of the recordings were repeated hourly up to 3 hours after the initial intake. All the measurements were done at the same time of day (starting at 9 a.m. in the morning). The EEG was recorded in monopolar mode from 17 surface electrodes according to the international 10/20, with A2 as a reference electrode and a 200-Hz sampling frequency (digital EEG device [Grass Technologies, West Warwick, RI, USA] with Telescan version 2.0 [Laxtha Inc., Daejeon, Korea]). Electrode attachment areas were designated as F (frontal), T (temporal), P (parietal), O (occipital), or C (central) depending on their brain surface locations. Electrodes were cleaned with an alcohol swab to eliminate sweat, oil, and cosmetics, and metallic accessories such as necklaces, rings, and watches were removed. To minimize artifacts caused by eye blinking, swallowing, and respiration, the recording procedure was controlled by a professional investigator, and the subjects were asked for their cooperation during the measurement. The signals from

Table 1. Baseline Characteristics of Subjects at Entry into Study

	Total subjects (MMSE-K 21–26)			*Subgroup (MMSE-K 21–23)*		
	LGNC-07 (n = *45*)	*Placebo* (n = *46*)	P*	*LGNC-07* (n = *11*)	*Placebo* (n = *9*)	P*
Gender						
Male (%)	13 (28.89)	12 (26.09)	.7647†	1 (9.09)	1 (11.11)	1.0000†
Female (%)	32 (71.11)	34 (73.91)		10 (90.91)	8 (88.89)	
Age (years)	57.58 ± 9.45	56.28 ± 9.92	.5253	59.36 ± 8.61	57.89 ± 9.31	.7174
Height (cm)	160.52 ± 6.68	159.69 ± 6.73	.5593	158.00 ± 5.73	158.89 ± 8.65	.7860
Weight (kg)	62.41 ± 7.71	59.33 ± 8.79	.0830	60.27 ± 9.07	57.67 ± 10.23	.5536
Blood pressure (mm Hg)						
Systolic	121.14 ± 12.62	123.04 ± 11.90	.4625	125.00 ± 15.09	128.89 ± 15.37	.5854
Diastolic	79.32 ± 8.73	79.78 ± 9.06	.8052	82.00 ± 9.19	82.22 ± 9.72	.9597
Pulse (rate/minute)	65.89 ± 6.12	66.83 ± 7.60	.5208	66.30 ± 5.89	68.00 ± 9.22	.6343
Temperature (°C)	36.54 ± 0.19	36.57 ± 0.18	.4914	36.60 ± 0.16	36.52 ± 0.19	.3447
Respiration (rate/minute)	19.95 ± 0.30	19.96 ± 0.29	.9750	20.00 ± 0.00	19.78 ± 0.67	.3466
MMSE-K	24.49 ± 1.32	24.85 ± 1.53	.2359	22.55 ± 0.52	22.11 ± 0.93	.2027
GDS						
Stage 2(%)	9 (20.93)	11 (24.44)	.6942†	2 (18.82)	0 (00.00)	.4789†
Stage 3(%)	34 (79.07)	34 (75.56)		9 (81.82)	9 (100.00)	

All data except gender and Global Deterioration Scale (GDS) are represented as mean ± SD values.

*P values by independent two-sample Student's t test unless otherwise noted.

†P values by χ^2 test.

LGNC-07, combination of green tea extract and L-theanine; MMSE-K, Mini Mental Scale Examination-K.

all electrodes underwent fast Fourier transformation, and data from 0.6 Hz to 50 Hz were analyzed by Telescan & Complexity software (Laxtha Inc.). The frequency bands were divided into four bands: delta (0–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz). The relative theta power spectrum, which is the ratio of the theta power spectrum to the sum of the total power spectrum (theta–gamma), was analyzed to determine the cognition enhancing effect of the treatment. Color-coding of maps was obtained by transforming the difference in power spectrum of hourly measurements from the baseline into RGB (red–green–blue) mode pictures.

Statistical analysis

Data are expressed as mean ± SD values in the tables and mean ± SE values in the figures. Baseline characteristics of the two subject groups were analyzed by an independent two-sample Student's t test and a χ^2 test. The statistical significance of differences within the groups was determined by a paired-sample Student's t test, and an independent two-sample Student's t test was used to determine the difference between the groups. To analyze data from stratified subjects whose MMSE-K was between 21 and 23, a nonparametric Wilcoxon rank sum test was used to determine the statistical difference. Differences were considered significant when $P < .05$.

RESULTS

Subject characteristics

The baseline clinical and mental characteristics of subjects involved in this study are presented in Table 1. Ninety-one subjects (25 men and 66 women) were randomly divided into treatment ($n = 46$) and placebo ($n = 45$) groups. There were no significant differences between the groups in gender, age, height, weight, blood pressure, or pulse rate.

MMSE-K and GDS scores, which represent the baseline cognitive functions of the participants, showed no significant difference between the two groups. There was no significant adverse events observed during the study, and blood level analyses remained in the normal range (Table 2).

Cognitive function tests

Verbal and visuospatial memory. A Rey–Kim memory test was conducted to assess the effect of LGNC-07 on verbal and visuospatial memory. Both LGNC-07 and placebo groups had increased MQ in the Rey–Kim memory test after 16 weeks, but there was no significant difference between the two groups. However, LGNC-07 marginally improved K-A (delayed recognition) at the 8-week time point ($P = .0572$ vs. placebo) (Table 3). Interestingly, in the stratified subjects with MMSE-K scores of 21–23 LGNC-07 showed significant MQ increase at 16 weeks ($P = .0478$ vs. placebo) (Table 4 and Fig. 1). In addition, LGNC-07 improved K-C (immediate recall) ($P < .01$ vs. before treatment) and K-C (delayed recall) ($P < .01$ vs. before treatment) after 16 weeks in comparison with the baseline measurements, whereas the placebo did not show any time course improvement. However, there was no significant difference between the two groups (Table 4).

Attention. The Stroop color–word reading test was conducted, and the correct count of word/color readings were taken as an indicator of frontal cortex lobe function and selective attention. Correct count of Stroop word reading did not increase at 16 weeks in either treatment. Stroop color reading was improved only by LGNC-07 ($P = .0099$ vs. before treatment) in comparison with the baseline measurements (Table 3). However, in the participants with MMSE-K scores of 21–23, LGNC-07 treatment led to a significant increase in Stroop word reading at 8 weeks ($P = .0306$ vs. placebo) (Table 4 and Fig. 1). Furthermore, Stroop color reading was improved by LGNC-07 at 16 weeks ($P = .0133$ vs. before treatment) in comparison with the baseline measurements, whereas there was no significant effect from the placebo, suggesting that LGNC-07 might also modulate color reading (Table 4).

Table 2. Results of Laboratory Tests for Safety

	Before treatment					*16 weeks*				
	LGNC-07 (n = *45*)		*Placebo* (n = *46*)			*LGNC-07* (n = *45*)		*Placebo* (n = *46*)		
Parameter	*Mean*	*SD*	*Mean*	*SD*	P*	*Mean*	*SD*	*Mean*	*SD*	P*
ALT (U/L)	23.53	17.38	21.07	11.87	.4323	28.98[a]	23.56	24.67	17.92	.3286
AST (U/L)	24.20	9.72	23.11	6.75	.5363	27.67[b]	11.57	24.24	7.53	.0988
γ-GTP (U/L)	25.42	15.99	20.41	11.93	.0933	28.44	23.04	19.89	8.26	.0226
BUN (mg/L)	15.16	3.46	15.00	4.85	.8527	15.31	4.33	16.23	5.37	.3714
Creatinine (mg/L)	0.89	0.17	0.88	0.16	.8566	0.91	0.18	0.90	0.16	.7500
RBCs ($10^6/mm^3$)	447.51	39.35	441.17	33.73	.4113	438.36[b]	42.14	434.24[a]	30.86	.5970
WBCs ($10^3/mm^3$)	61.51	13.29	62.50	18.50	.7700	60.36	15.93	59.24	16.29	.7700

*P values by independent Student's t test between LGNC-07 and placebo.

[ab]Values denote significant increase in 16 weeks by paired-sample t test: [a]$P < .05$, [b]$P < .01$.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; γ-GTP, γ-glutamyltransferase; RBC, red blood cell; WBC, white blood cell.

Table 3. Neuropsychological Tests of LGNC-07 with All Subjects

	Change at 8 weeks			*Change at 16 weeks*		
	LGNC-07 (n = *45*)	*Placebo* (n = *46*)	P*	*LGNC-07* (n = *45*)	*Placebo* (n = *46*)	P*
Rey–Kim memory test						
MQ	5.89 ± 9.16^{c}	4.50 ± 8.82^{c}	.4633	10.60 ± 10.51^{c}	11.26 ± 9.10^{c}	.7490
Verbal learning test						
K-A: Trial 1	1.18 ± 2.75^{b}	1.63 ± 2.24^{c}	.3914	1.73 ± 2.96^{c}	2.37 ± 2.28^{c}	.2539
K-A: Trial 2	1.07 ± 2.38^{c}	1.35 ± 2.86^{c}	.6119	1.38 ± 2.58^{c}	2.50 ± 3.32^{c}	.0755
K-A: Trial 3	0.78 ± 2.20^{a}	0.72 ± 2.05^{a}	.8927	1.22 ± 2.63^{c}	1.28 ± 1.93^{c}	.9010
K-A: Trial 4	1.47 ± 2.97^{c}	1.13 ± 2.38^{c}	.5521	1.87 ± 2.97^{c}	1.13 ± 1.83^{c}	.1597
K-A: Trial 5	0.87 ± 2.37^{a}	0.72 ± 2.10^{a}	.7513	0.91 ± 2.48^{a}	0.48 ± 2.05	.3669
K-A (delayed recall)	1.04 ± 2.39^{b}	0.98 ± 2.62^{a}	.9002	1.60 ± 2.57^{c}	1.87 ± 2.07^{c}	.5828
K-A (delayed recognition)	1.09 ± 3.04^{a}	-0.11 ± 2.89	.0572	1.51 ± 3.17^{c}	0.59 ± 2.38	.1186
Complex figure test						
K-C (copy)	-0.09 ± 3.69	0.46 ± 3.37	.4635	0.91 ± 2.77^{a}	1.24 ± 3.33^{a}	.6114
K-C (immediate recall)	1.38 ± 2.68^{c}	1.70 ± 2.46^{c}	.5569	2.53 ± 2.74^{c}	2.83 ± 2.89^{c}	.6212
K-C (delayed recall)	2.83 ± 2.89^{c}	1.70 ± 2.48^{c}	.5987	2.47 ± 2.87^{c}	2.85 ± 2.89^{c}	.5292
Stroop color–word test						
Word reading	3.31 ± 11.90	0.37 ± 2.98	.1078	2.47 ± 12.25	0.67 ± 2.41	.3329
Color reading	5.47 ± 19.34	2.72 ± 15.98	.4612	9.33 ± 23.22^{b}	4.74 ± 18.07	.2945

Data are represented as differences from baseline, indicated as mean ± SD values.

*P values by independent two-sample Student's t test for difference from baseline for LGNC-07 versus placebo.

[abc]Values denote significant increase after treatment at each time point by paired-sample t test: [a]$P < .05$, [b]$P < .01$, [c]$P < .005$.

MQ, memory quotient.

EEG

Twelve subjects were randomly chosen from each group, making up a total of 24 subjects who participated in the EEG study. There was no significant difference in demographic and neuropsychological characteristics such as gender, age, height, weight, blood pressure, pulse rate, MMSE-K score, or GDS stage (Table 5).

Subjects took four capsules of treatment or placebo at a time to evaluate the short-term effect of treatment. Differences between the baseline and hourly measurements were used to assess the activity of the theta power spectrum after LGNC-07 intake. Results are presented as a time course change of theta activity from all electrodes (Fig. 2) and as a color-coded map (Fig. 3) during the three recording conditions (eyes-open, eyes-closed, and reading). Data analysis

Table 4. Effect of LGNC-07 on Subjects with Mini Mental Scale Examination-K Scores of 21–23

	Change at 8 weeks			*Change at 16 weeks*		
	LGNC-07 (n = *11*)	*Placebo* (n = *9*)	P*	*LGNC-07* (n = *11*)	*Placebo* (n = *9*)	P*
Rey–Kim memory test						
MQ	8.82 ± 10.39^{a}	4.78 ± 10.44	.5176	14.18 ± 10.56^{c}	6.00 ± 7.19^{a}	.0478
Verbal learning test						
K-A: Trial 1	1.91 ± 2.26^{a}	1.44 ± 2.70	.6093	3.18 ± 1.54^{c}	3.00 ± 2.35^{b}	.8465
K-A: Trial 2	0.73 ± 2.37	0.56 ± 1.59	.7579	1.09 ± 2.81	1.67 ± 1.73^{a}	.7558
K-A: Trial 3	1.18 ± 2.18	-0.22 ± 2.99	.2265	1.91 ± 2.55^{a}	-0.22 ± 2.99	.8472
K-A: Trial 4	0.82 ± 1.72	0.44 ± 1.81	.6151	1.00 ± 2.24	0.89 ± 1.90	.7280
K-A: Trial 5	0.73 ± 2.76	1.33 ± 2.29	.7280	-0.18 ± 1.99	0.78 ± 2.68	.4900
K-A (delayed recall)	1.18 ± 2.79	0.78 ± 3.70	.7877	2.64 ± 3.04^{a}	1.89 ± 2.26^{a}	.2811
K-A (delayed recognition)	1.45 ± 4.03	1.73 ± 4.05	.4424	0.89 ± 3.59	1.33 ± 2.40	.7309
Complex figure test						
K-C:Copy	0.45 ± 5.01	1.11 ± 3.69	.6469	2.18 ± 3.37	1.00 ± 2.74	.2982
K-C:Immediate recall	0.91 ± 3.21	1.78 ± 1.99^{a}	.4605	3.64 ± 2.91^{c}	1.78 ± 3.67	.3003
K-C:Delayed recall	1.82 ± 3.92	1.78 ± 2.49	.9691	3.64 ± 2.94^{c}	1.89 ± 3.41	.2516
Stroop color–word test						
Word reading	3.18 ± 7.04	-0.44 ± 1.88	.0306	1.91 ± 6.27	0.11 ± 2.57	.6734
Color reading	8.27 ± 20.53	0.89 ± 18.42	.5183	17.18 ± 18.99^{a}	6.00 ± 22.58	.1596

Data are represented as differences from the baseline, indicated as mean ± SD values.

*P value by Wilcoxon rank sum test for LGNC-07 versus placebo.

[abc]Values denote significant increase after treatment at each time point by paired-sample t test: [a]$P < .05$, [b]$P < .01$. [c]$P < .005$.

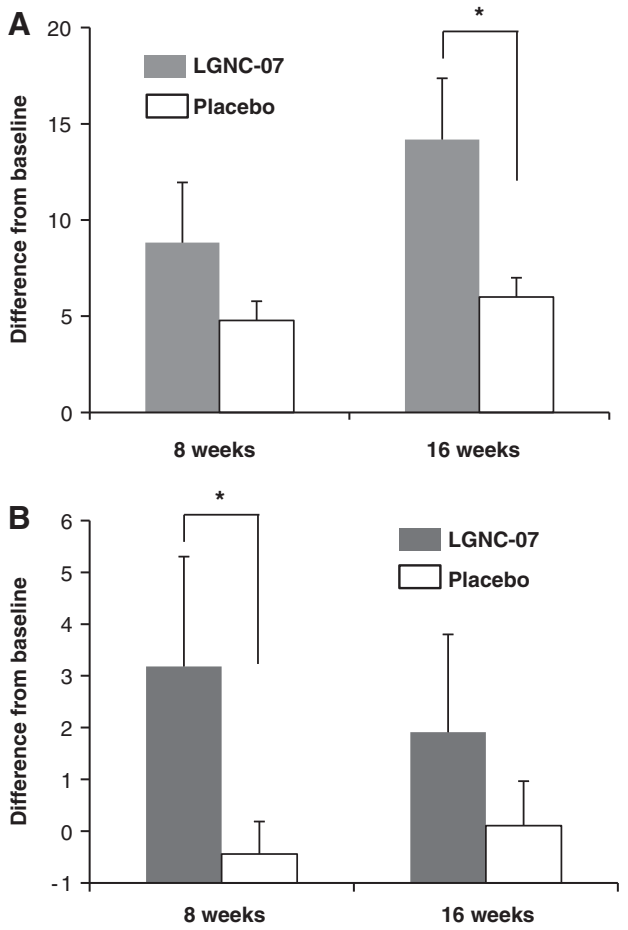

FIG. 1. LGNC-07 improves cognitive function (memory/attention) of the subjects with MMSE-K scores of 21–23. Subjects whose MMSE-K scores were between 21 and 23 (LGNC-07, $n = 11$; placebo, $n = 9$) showed memory and attention improvement after LGNC-07 intake. (**A**) Rey–Kim MQ (memory) and (**B**) Stroop word reading (attention) increased significantly in 16 weeks ($P = .0478$ vs. placebo) and 8 weeks ($P = .0306$ vs. placebo), respectively. Data are mean ± SE values. *$P < .05$.

was based on 23 subjects, as one of the subjects from the LGNC-07 group was excluded because of a baseline artifact during measurement.

During reading, changes in the theta spectrum tended to increase over time in the frontal area after LGNC-07 treatment, reaching statistical significance at 2 and 3 hours at F7 and at 2 hours at F8 (Fig. 2). Significant increases were also observed in the temporal (T6 in 3 hours), parietal (P3 and Pz in 2 hours), and central (Cz in 2 hours) areas. In the eyes-open state, the theta spectrum increased significantly at 3 hours in the temporal (T5, T6), parietal (P3), and occipital (O1, O2) areas. In contrast to the eyes-open and reading states, theta activity showed a tendency to decrease in the eyes-closed state in the LGNC-07 group, especially in areas other than the frontal area (Fig. 2).

A color-coded brain map was used to show the proportional changes of theta power in all the brain areas, which is coded as red for an increase and blue for a decrease (Fig. 3). It is evident that LGNC-07 started increasing theta waves at 1 hour and that its activity lasted for up to 3 hours during the eyes-open state. In the reading state, the topographical pattern is similar between the two groups at 1 hour; however, theta waves were distinctly increased by LGNC-07 treatment after 2 and 3 hours.

DISCUSSION

The importance of early diagnosis and treatment of SMCs has recently been emphasized because subjects with SMCs have a higher risk of dementia[31] and SMCs are central to diagnosing MCI, which is thought to be an intermediate state or the onset of AD. Studies show that 12% of MCI subjects progress to AD within 1 year and 80.5% within 6 years.[32,33] MCI is characterized as memory impairment with a conservation of daily life activities and a 0.5 on the Clinical Dementia Rating scale.[34] MCI is divided into amnestic MCI, multiple domains slightly impaired MCI, and single non-memory domain MCI. Petersen *et al*.[5] proposed following criteria for amnestic MCI: (1) memory complaints, preferably corroborated by an informant, (2) impaired memory function for age and education, (3) preserved general cognitive function, (4) intact abilities of daily living, and (5) not demented. Amnestic MCI has a higher rate of AD conversion than other types of MCI. Various rating scales and neuropsychological tests have

Table 5. Subject Characteristics for Electroencephalography

	LGNC-07 (n = *12*)	*Placebo* (n = *12*)	P*
Gender			
Male (%)	2 (16.67)	2 (16.67)	1.0000†
Female (%)	10 (83.33)	9 (75.00)	
Age (years)	56.75 ± 8.61	60.67 ± 9.35	.2976
Height (cm)	158.18 ± 7.69	158.73 ± 6.89	.8627
Weight (kg)	62.91 ± 8.81	58.36 ± 8.44	.2310
Blood pressure (mm Hg)			
Systolic	118.33 ± 13.37	120.83 ± 9.96	.6087
Diastolic	76.67 ± 10.73	78.33 ± 8.35	.6752
Pulse (rate/minute)	65.00 ± 5.69	65.67 ± 7.95	.8154
Body temperature (°C)	36.57 ± 0.22	36.59 ± 0.12	.7375
Respiration (rate/minute)	19.83 ± 0.58	20.00 ± 0.00	.3388
Mini-Mental Scale Exam	24.83 ± 0.94	24.58 ± 1.78	.6726
GDS			
Stage 2(%)	2 (16.67)	3 (25.00)	1.0000†
Stage 3(%)	10 (83.33)	9 (75.00)	

All data except gender and GDS are represented as mean ± SD values.
*P values by independent two-sample Student's t test otherwise noted.
†P values by χ^2 test.

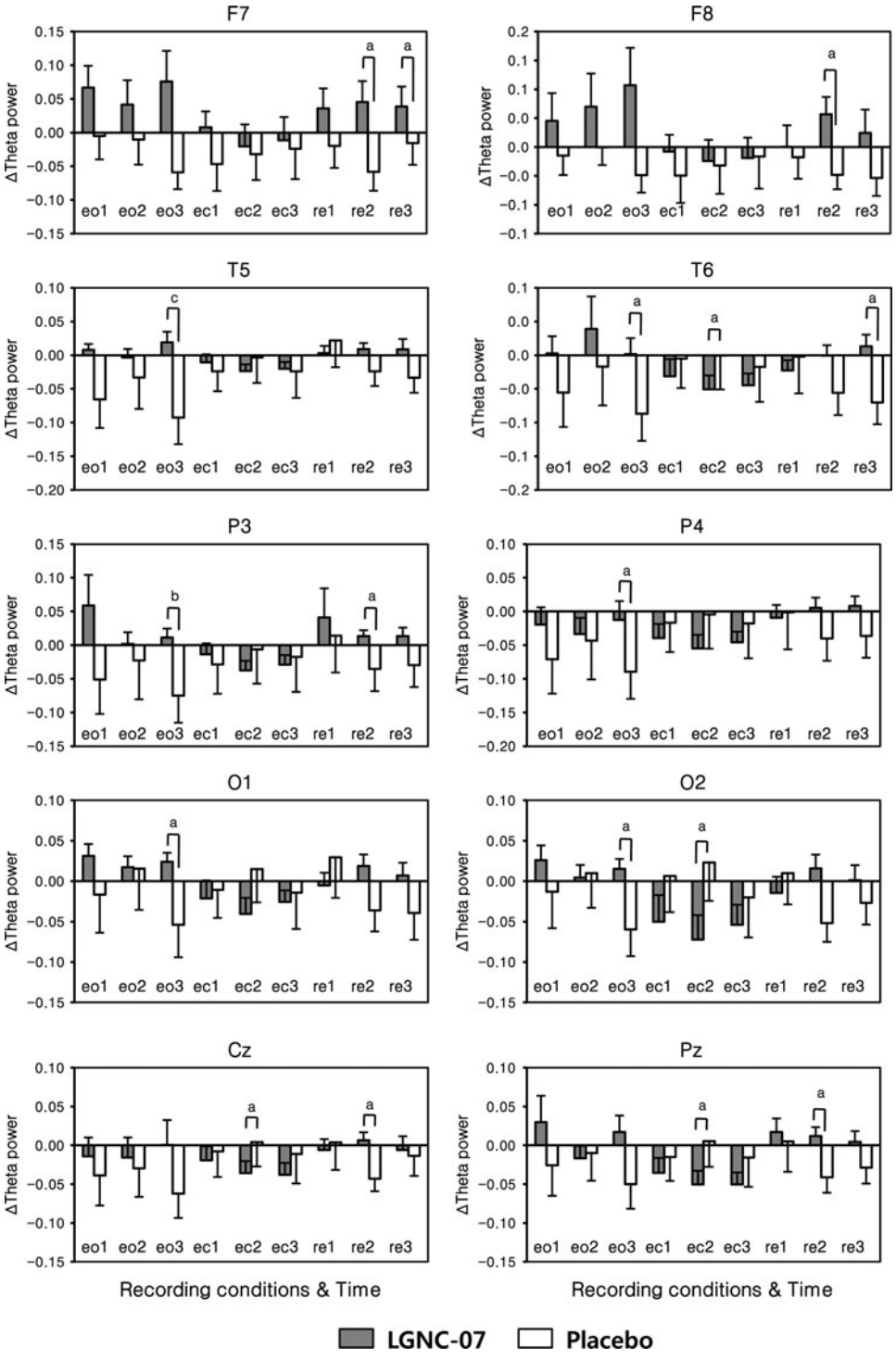

FIG. 2. Changes in theta waves for 3 hours after LGNC-07 intake under the recording conditions in eyes-open (eo), eyes-closed (ec), and reading states (re). Differences between baseline and each time point from 23 subjects (LGNC-07, $n = 11$; placebo, $n = 12$) were presented in the frontal (F), temporal (T), parietal (P), occipital (O), and central (C) areas of the brain surface. Data are mean ± SE values. $^{a}P < .05$, $^{b}P < .01$, $^{c}P < .005$ by Wilcoxon signed rank sum test.

been instrumented to characterize MCI. More than one method needs to be adopted because no single test can cover all the features of this clinical condition. Stages 2 and 3 of GDS are categorized as MCI, and neuropsychological tests that measure new learning, delayed recall, and executive function are recommended to define MCI.[5]

Several independent clinical reports showed the memory improvement potential of each ingredient that makes up LGNC-07 (GTE and L-theanine). Kim and Yoon[35] reported that GTE containing γ-aminobutyric acid showed a tendency to increase word memory count, and Ng *et al.*[36] showed a decrease in cognitive deficiency in elderly subjects over 55 years old. L-Theanine- and theogallin-enriched green tea consumption increased theta brain waves, which suggests a higher level of mental performance.[19] In addition, the combined treatment of L-theanine and caffeine increased

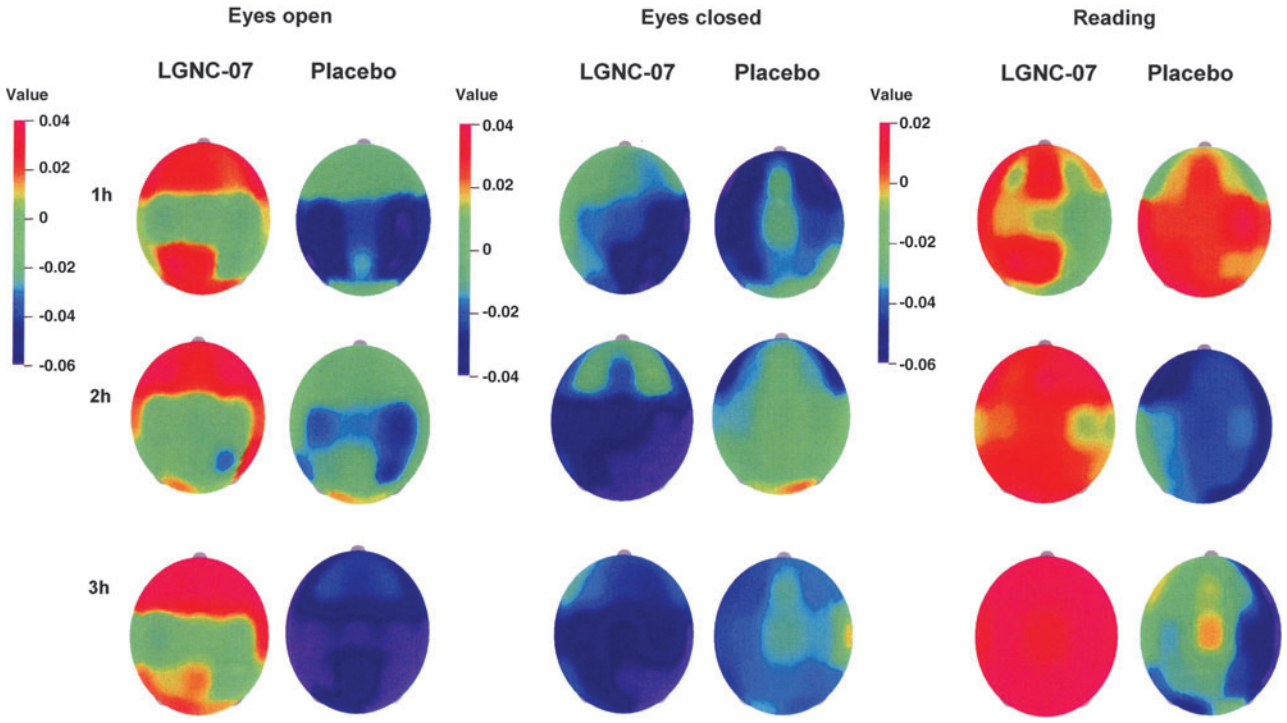

FIG. 3. Topographic diagram of theta wave measured for 3 hours after LGNC-07 intake. Average changes in theta wave from 12 subjects are indicated in the RGB (red–green–blue) mode. Theta waves increased significantly during the eyes-open and reading states.

the hit rate of attention tasks using a cathode ray tube monitor, which indicates an improvement in the subject's attention.[37] However, there have been no interventional studies using a combination treatment of GTE and L-theanine and assessing cognitive performance.

In this study, we investigated the effect of LGNC-07 on cognitive function in MCI subjects with MMSE-K scores of 21–26 using a neuropsychological test battery. The Rey–Kim memory test was used to determine memory improvement, and the Stroop color–word reading test was used to measure attention. The Rey–Kim memory test is an instrument used to measure verbal memory (K-AVLT) and visuospatial memory (K-CTF). K-AVLT is designed to measure memory registration, retention, and retrieval, which consists of repeated trials as well as immediate and delayed recall. LGNC-07 showed a marginal increase in delayed recognition at 8 weeks, which suggests that the potential mechanism of its effect on memory improvement is through memory retrieval. What is interesting in this study is that the Rey–Kim MQ increased significantly in stratified subjects with MMSE-K scores of 21–23. The Rey–Kim MQ is one of the most important criteria in the test and is useful because it is presented as a sum of the conversion scores, reflecting overall performance in memory and learning. The Rey–Kim memory test is a sensitive tool to measure memory impairment; therefore, it is highly probable that it is more sensitive for detecting changes in cognitive function in relatively severe cognitive impairment groups with MMSE-K scores between 21 and 23.

Attention was assessed by the Stroop color–word reading test, which is a cognitive test used to assess frontal function and selective attention in AD subjects. LGNC-07 showed a tendency for the correct word reading count to increase after 16 weeks, although the difference was not statistically significant. Color reading increased significantly over time during LGNC-07 treatment but was not significantly different from the placebo. It is notable that LGNC-07 treatment led to a significant increase at 8 weeks compared with the placebo in the stratified group, which is consistent with the improvement in cognitive function in subjects with MMSE-K between 21 and 23.

Increased theta activity during the eyes-open and reading states supports the findings from the neuropsychological test, showing that LGNC-07 increases cognitive functions such as memory and attention. Although other investigators believe that the frontal region is the main contributor to cognitive function because of its condensed neurotransmitter receptors, it is also noteworthy that the theta spectrum also increased in the parietal and occipital areas in our study. Based on the previous report, ingestion of a drink containing gingko and ginseng extract increases theta activity in brain regions other than the frontal areas.[20] Therefore, it might be premature to decide that the frontal region is the only area to be studied. Moreover, Jacobs *et al.*[38] suggested that increased theta activity in the left-parietal and central regions is related with memory retrieval and decision-making.

There is a clear need for further studies to investigate the cognitive improvement effect of LGNC-07. These studies

should have increased numbers of study subjects with various degrees of cognitive deficits. In particular, the finding that the subgroup whose MMSE-K was between 21 and 23 showed a better response to LGNC-07 needs to be carefully considered. Several different neuropsychological tests may be needed to elucidate how LGNC-07 regulates cognitive function. Additionally, a nutritional perspective of the treatment could be evaluated with baseline nutritional information measurements.

In conclusion, this study showed that LGNC-07, a combination of GTE and L-theanine, improved cognitive function by increasing memory and attention in MCI subjects whose MMSE-K was between 21 and 23. LGNC-07 also increased theta activity during active mental states (eyes-open and reading) as shown by EEG, further supporting its effect on the neuropsychological tests. As a natural ingredient with a long history of consumption, LGNC-07 should be considered as a potential nutraceutical candidate for enhancing cognitive performance.

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AUTHOR DISCLOSURE STATEMENT

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