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ORIGINAL ARTICLE

# Oral intake of γ-aminobutyric acid affects mood and activities of central nervous system during stressed condition induced by mental tasks

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**Abstract** γ-Aminobutyric acid (GABA) is a kind of amino acid contained in green tea leaves and other foods. Several reports have shown that GABA might affect brain protein synthesis, improve many brain functions such as memory and study capability, lower the blood pressure of spontaneously hypertensive rats, and may also have a relaxation effect in humans. However, the evidence for its mood-improving function is still not sufficient. In this study, we investigated how the oral intake of GABA influences human adults psychologically and physiologically under a condition of mental stress. Sixty-three adults (28 males, 35 females) participated in a randomized, single blind, placebo-controlled, crossover-designed study over two experiment days. Capsules containing 100 mg of GABA or dextrin as a placebo were used as test samples. The results showed that EEG activities including alpha band and beta band brain waves decreased depending on the mental stress task loads, and the condition of 30 min after GABA intake diminished this decrease compared with the placebo condition. That is to say, GABA might have alleviated the stress induced by the mental tasks. This effect also corresponded with the results of the POMS scores.

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**Keywords** γ-Aminobutyric acid · Electroencephalogram · Acute stress · Profile of Mood States

**Abbreviations**

| | |
|---|---|
| GABA | γ-Aminobutyric acid |
| EEG | Electroencephalogram |
| CNS | Central nervous system |
| ANS | Autonomic nervous system |
| CgA | Chromogranin A |
| IgA | Immunoglobulin A |
| AT | Arithmetic mental task |
| DT | Auditory oddball target detection task |
| POMS | Profile of Mood States |
| VAS | Visual analogue scales |
| 5-HT | 5-Hydroxytryptamine |

## Introduction

Recently, growing attention has been paid to eating natural, minimally processed, nutritional, and healthful foods as a way to live a healthier life. γ-Aminobutyric acid (GABA) became famous for its functions in the central nervous system (CNS) and autonomic nervous system (ANS). GABA is a kind of amino acid widely distributed in mammals, crustaceans, microorganisms, and many plants such as green tea leaves, tomatoes, and many other foods. It is known as an inhibitory transmitter compound in vertebrates and is present at a high concentration in the CNS (Roberts and Frankel 1950; Otsuka et al.1966; Curtis and Johnston 1974; Hefft et al. 2002).

Several animal experiments have demonstrated that the administration of GABA increased the concentrations of plasma growth hormone and the rate of protein synthesis in the brain (Tujioka et al. 2007, 2009), improved many brain

functions such as memory and study capability, and lowered the blood pressure of spontaneously hypertensive rats (Yokogoshi and Kobayashi 1998, Mitsushima et al. 2002, Lyou and Yokogoshi 2004, Yamakoshi et al. 2007).

The effect of orally administered GABA on human ANS activities was also reported recently. Fujibayashi et al. (2008) suggested that 30 mg GABA increases overall ANS and parasympathetic nervous system activities and thus may induce relaxation effects. Nakamura et al. (2009) studied the psychological stress-reducing effect of chocolate enriched with GABA (28 mg) using stress induced by an arithmetic task and measuring the changes of heart rate variability and salivary chromogranin A (CgA).They found that taking GABA-enriched chocolate led to a quick recovery to the normal state from the stressful state. Also the CgA value measured after the task in those taking GABA chocolate did not increase in comparison with that measured before ingestion. From these results, GABA was considered to have a psychological stress-reducing effect.

Abdou et al. (2006) investigated the effect of 100 mg GABA intake on relaxation and immunity during stress by evaluating the electroencephalogram (EEG) and immunoglobulin A (IgA) levels. In their first experiment, EEG recordings were obtained for 5 min with 13 participants resting quietly with closed eyes before and after 30- and 60-minute resting sessions. Their EEG results indicated that GABA increased alpha waves and decreased beta waves. In their second experiment, saliva was sampled from eight participants before, at the middle and at the end of a suspended bridge, and the GABA intake group showed higher IgA levels. With these they concluded that GABA could work effectively as a natural relaxant, and its effects could be seen within 1 h of its administration to induce relaxation, diminish anxiety, and enhance immunity under stress conditions. However, we found in our preliminary experiments that many of the participants fell asleep during the resting sessions and also during the EEG measurements when they closed their eyes for longer than 2 min. That is to say, it is possible that this might have been the case in the EEG study reported by Abdou et al., especially if the subjects were in a drowsy state. Our concern is to make it clear whether the oral intake of GABA has a mood-improving effect on human CNS activity during wakefulness; thus, a new experimental design was needed to keep the participants awake during the study. We also found it difficult to control the subjects' mental activities such as thinking of other things during the rest sessions, even though the participants were asked to avoid thinking. These mental activities might have influenced the EEG activities differently depending on what the participants were thinking about, which would result in a bias in the EEG results. We considered that shortening the time allotted for EEG measurement with the participants' eyes closed to 1 min, and having them perform a mental task during the long-waiting resting session could solve this problem.

With respect to the mental task, Kanehira et al. (2011) observed that 25 or 50 mg GABA taken orally had an anti-fatigue effect on the salivary secretion levels of chromogranin A (CgA) and cortisol in nine participants who were diagnosed with chronic fatigue. A 15-min arithmetic task (Uchida–Kraepelin psychodiagnostic test) was used as a fatigue load between the saliva samplings. They found that the intake of GABA-containing beverages, especially those containing 50 mg of GABA, resulted in significantly lower levels of CgA and cortisol, suggesting that GABA intake had an anti-fatigue effect. Meanwhile, because no EEG was recorded in their study, it remains uncertain how the GABA intake influences the brain electric activities under the mental task stress load.

In this study, we assessed the effects of GABA intake on CNS activities in healthy people under mental task stress loads by measuring EEG, and we evaluated subjects' Profile of Mood States (POMS) scores and the visual analogue scale (VAS) scores as subjective ratings on mental state.

## Materials and methods

The experiment in this study was approved by the Research Ethics Committee in the University of Shizuoka, and was carried out in accordance with the Declaration of Helsinki.

### Participants

Sixty-three healthy volunteers (28 males, 35 females, ages 24.5 ± 4.0 years old, height 164.4 ± 9.0 cm, weight 58.1 ± 11.0 kg) participated in the experiment individually at a similar time of day with an interval of 24 h. All participants were requested to avoid eating or drinking except for water intake beginning 3 h before the start of each trial.

### Treatment

A single blind, cross-over, randomized, placebo-controlled design was used in this study. Two separate trials were performed in which the participants orally took either GABA or dextrin as a placebo (100 mg each, Pharma Foods International Co. Ltd) each day. All sample capsules were taken with 250 ml of warm water at 25°C.

### Stress load task

An arithmetic mental task (AT) lasting 10 min and an auditory oddball target detection task (DT) lasting 5 min were imposed together in one session of stress loading.

Three sessions were performed at around 10, 40, and 70 min after the administration of the test sample, respectively, within each trial. The AT required participants to add two numbers (one appeared randomly from among the numbers 1 to 9 and another from 1 to 19) which were concurrently displayed on a PC monitor. The answers were asked to be entered through a keyboard as quickly and accurately as possible. In the DT, the participants were required to click the left button of a mouse device as quickly as possible only in response to target stimuli (single tone, 2,000 Hz lasting for 0.1 s) that occurred infrequently and irregularly within a series of standard stimuli (single tone, 1,000 Hz, lasting 0.1 s).

### Subjective assessment

The POMS and the VAS, used for subjective ratings on mood state, were completed right after the intakes for baseline data and after all of the three mental task sessions were finished.

The short version of POMS was held to assess distinct affective mood states. POMS is a popular tool used widely among psychologists and scientists from many other fields. Six identifiable mood or affective states: tension-anxiety (T-A), depression-dejection (D), anger-hostility (A-H), vigor-activity (V), fatigue-inertia (F), and confusion-bewilderment (C) can be measured and were used for analysis in this study.

VAS comprises five scales including the feelings of fatigue, relaxation, arousal, pressure, and tension. At the end of each trial, scales for the feelings of annoyance about DT and AT were completed.

### Measurement

Active electrodes were attached for EEG recording at five locations: F3, F4, Pz, O1, and O2 according to the international 10/20 system. Electrooculogram (EOG) was recorded at the left eye supra- and infra-orbitally for monitoring the ocular movements. EEG and EOG data were amplified and A/D converted by a versatile amplification unit (polymate AP1132, TEAC Corporation), and FFT was transferred offline using VitalTracer and ATAMAPII (KISSEI COMTEC CO. LTD). Filters were set for EEG at high pass of 0.016 Hz and low pass of 60 Hz, for EOG at high pass of 0.16 Hz and low pass of 15 Hz. The sampling rate was 1,000 Hz. Absolute EEG band power was calculated in the alpha ($\geq$8, <13 Hz) and beta ($\geq$13, <30 Hz) bands. Data with artifacts such as ocular or body movements were excluded from further processing. The mean band power of the alpha and beta calculated from the first minute of each EEG measurement with the participant's eyes closed were used for the analysis.

### Procedure

Each participant was required to attend a total of two study days for about 2 h per day. Figure 1 shows the procedure of the experiment. Prior to the start of the experiment, all participants were given the opportunity to familiarize themselves with all of the stress load tasks. Experiments took place in a quiet room. The room temperature was 24.3 ± 1.5°C, and the humidity was 46.7 ± 10.0%. On the experiment day, the participant entered the room, was seated and rested for 15 min. During the resting time, electrodes were attached. After the rest, either GABA or placebo capsules were administered orally with a cup of water (250 ml, 25°C). Then a 4-minute EEG measurement session for baseline data took place, and the participant then filled out POMS and VAS forms. At around 11 min after the sample intake, the participant performed ATs for 10 min, then rested for 2 min, performed DTs for 5 min, and rested again for 2 min. This regimen was repeated after EEG measurements at 30, 60, and 90 min after intake. After the 4th session of POMS and VAS was completed, the electrodes were taken off, and the participant left the experiment room.

### Statistic analysis

Data were analyzed using IBM SPSS Statistics version 19. Non-parametric Friedman tests were performed to detect differences among the four time courses (i.e., 0, 30, 60, and

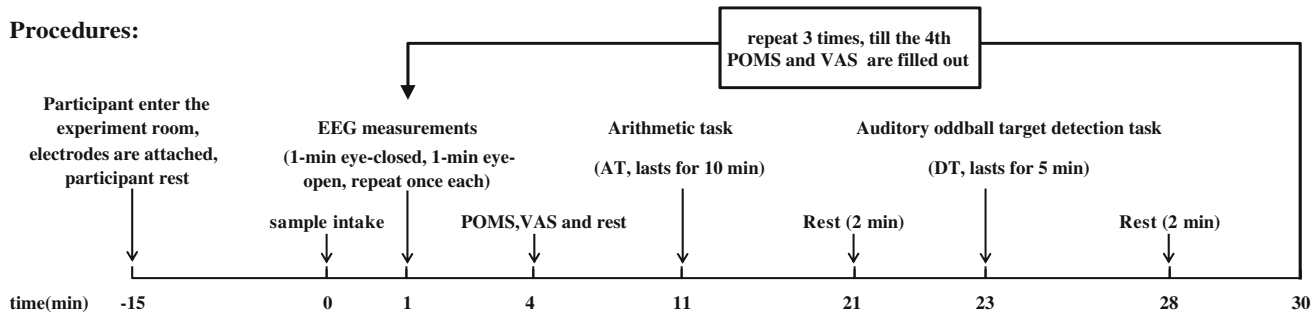


**Fig. 1** Procedures used in this study. EEG measurements and subjective assessments including POMS and VAS were performed a total of four times, i.e., 0, 30, 60, and 90 min after sample intake

90 min after intake). Wilcoxon signed-rank tests with Bonferroni correction were then carried out to evaluate the changes of each time course compared with the baseline, which was measured at 0 min after intake. Wilcoxon Signed-Rank tests were also performed for the comparisons between sample treatments.

## Results

### POMS

Figure 2 presents the POMS scores. The results showed that mental stress tasks decreased the V scores in both sample-treatment conditions compared with the baseline evaluation ($P < 0.01$), and GABA intake resulted in smaller decreases than in the placebo condition in all of the three measurement segments ($P < 0.05$).

### EEG

The EEG alpha band power in the left frontal area in the GABA treatment condition declined after each mental stress task session significantly ($P < 0.05$, 0.01, and 0.05 at 30, 60, and 90 min after intake, respectively). Similar alpha band power changes can be found in the placebo treatment condition in this area too ($P < 0.01$ at 30 and 60 min after intake). The alpha band power at 30 min after GABA intake showed a smaller decrease than in the placebo treatment ($P < 0.05$, Fig. 3, upper left).

The upper right graph shows similar results in the right frontal area. The alpha band power declined significantly in the GABA treatment condition ($P < 0.1$, 0.01, and 0.1 at 30, 60, and 90 min after intake). In the placebo treatment condition, the alpha band power also decreased at 30 and 60 min after the sample intake ($P < 0.01$ and 0.1, respectively). The alpha band power at 30 min after GABA intake showed a smaller decrease than in the placebo treatment ($P < 0.05$).

The lower left graph shows changes of the beta band power in the left frontal area. In both treatment conditions, the beta band power declined from 30 to 90 min after intake ($P < 0.01$ after each sample intake). It was found that this decrease of beta band power was smaller at 30 min after GABA intake than after the placebo treatment ($P < 0.05$).

The lower right graph shows changes of the beta band power in the right frontal area. In both sample-treatment conditions, the beta band power declined significantly ($P < 0.01$, 0.01, and 0.1 in GABA, and $P < 0.01$, 0.01, and 0.01 in the placebo intake condition 30, 60, and 90 min after intake, respectively). The EEG band power 30 min after placebo intake tended to be lower than that 30 min after GABA intake ($P < 0.1$), and was lower at 90 min after placebo intake than after GABA intake ($P < 0.05$).

We also calculated the alpha/beta ratios in each electrode location and applied a Wilcoxon signed-rank test between sample treatments, but no significant difference was found (data not shown).

### VAS

Changes from baseline in the scores for every item were analyzed by Wilcoxon signed ranks, but no significant difference between sample treatments could be found (data not shown).

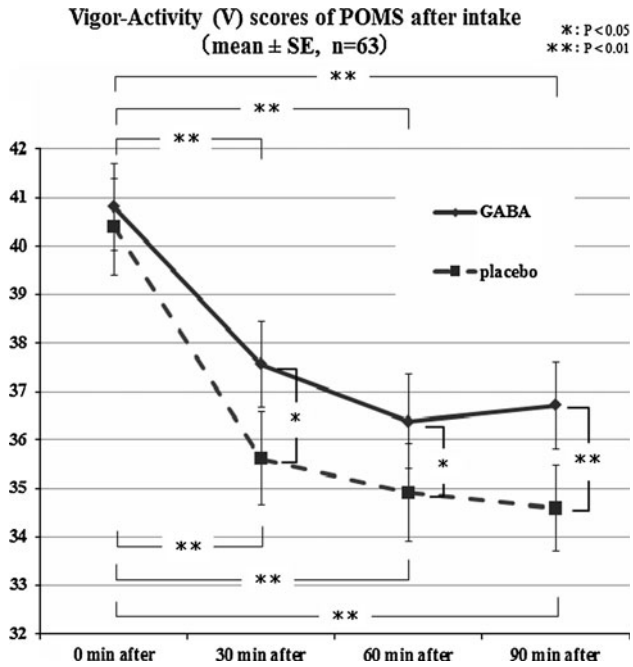


**Fig. 2** Vigor-activity (V) POMS scores after mental tasks and the results of comparisons by Wilcoxon signed-rank tests with Bonferroni correction

## Discussion

EEG activities including alpha band and beta band brain waves decreased from 30 to 60 min after intake (Fig. 3), indicating that the mental stress tasks used in our study might have reduced the overall brain activities from alpha band to beta band during the rest periods. On the other hand, at 30 min after GABA intake, this decrease diminished compared to the placebo condition.

Enhanced alpha oscillations have long been considered an attribute of relaxation beginning with Berger's study in 1929. Yet recently, alpha power has been reconceptualized as a mechanism for increasing signal-to-noise ratios within

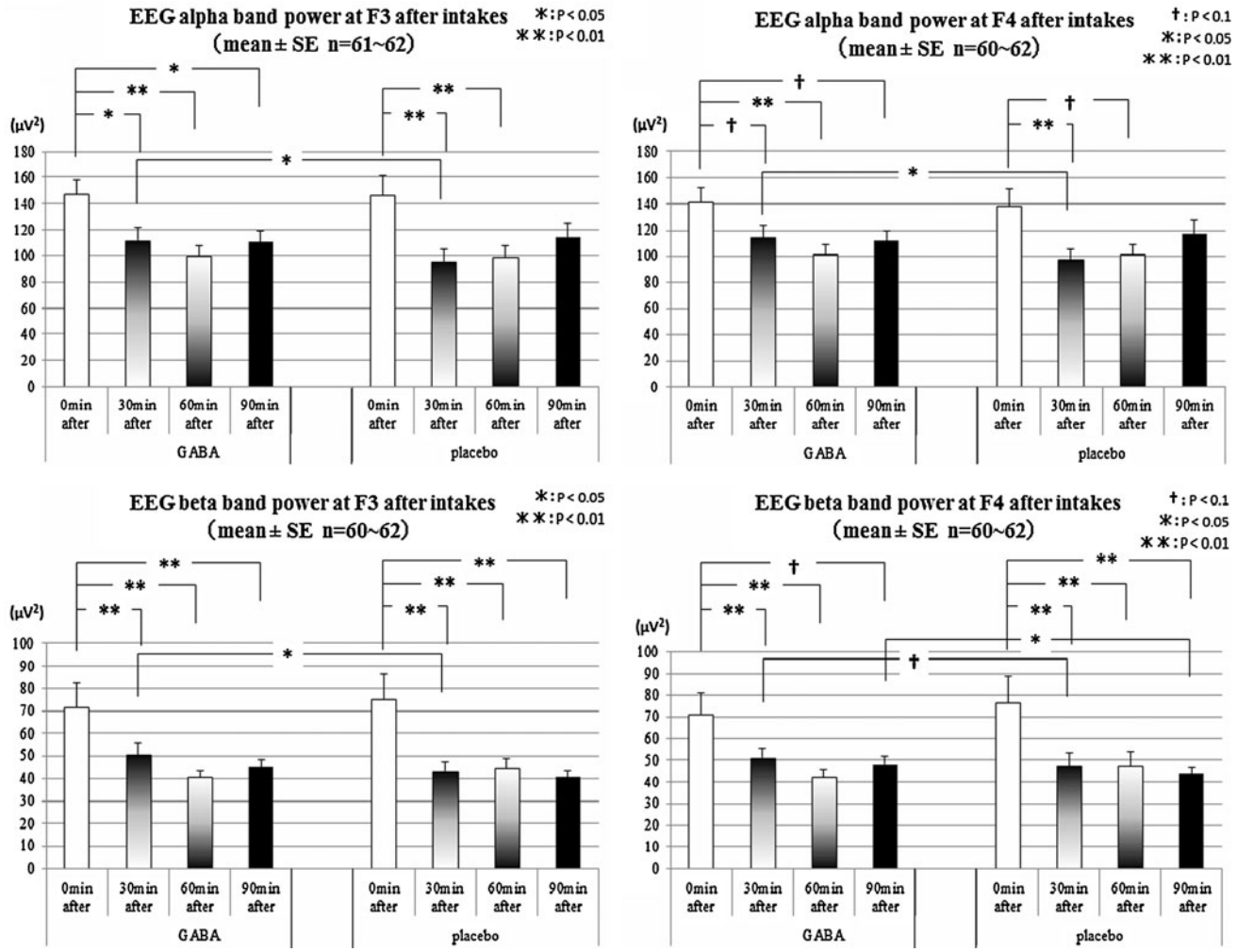


**Fig. 3** Mean power of EEG alpha band and beta band in the left and right frontal areas (F3 and F4). Comparisons were performed using Wilcoxon signed-rank tests with Bonferroni correction

the cortex by means of the inhibition of conflicting processes unnecessary to the task at hand: the greater the task demands, the more inhibition needed, and the greater the synchronization (Klimesch et al. 1999, 2000). Klimesch (1999) states that the reactivity in band power can be predicted from the amount of absolute power as measured during the resting state. A high level of alpha power is associated with a large amount of desynchronization during task performance. A large amount of upper alpha power in a reference interval preceding a task was related to both strong suppression of upper alpha power during the task and good performance.

Beta activity is closely linked to motor behavior and is generally attenuated during active movements associated with active, busy or anxious thinking and active concentration (Basar et al. 2000). Simultaneous occurrence of power activities in the alpha and lower beta bands can be observed when the events inducing the activity are relatively simple to process (Pfurtscheller and Lopes da Silva 1999).

From our EEG results, the decrease in both the alpha and beta band power measured in the intervals of the mental stress tasks might be considered to be a reaction to the stressful mental tasks. 30 min after GABA intake, the GABA caused this reaction to diminish. This finding agreed with the psychological stress-reducing effect of GABA intake reported in other studies using salivary stress markers, e.g., Nakamura et al. (2009) and Abdou et al. (2006). We noticed that at 30 min after intake the GABA concentration in the blood reached its peak in an animal study (Abdou et al. 2006). Thus, the effect of GABA intake was consistent with this timing. Meanwhile, no difference was found in the alpha/beta ratios between sample treatments, even though it has been reported that GABA increased alpha waves and decreased beta waves in a study by Abdou et al. (2006). This discrepancy might be due to the difference of the experimental design. In our study, mental stress tasks were additionally performed among the measurements to avoid the influence of different mental activities such as thinking of

different things unrelated to our study or falling asleep during the long waiting periods.

This anti-stress effect of GABA intake can also be proved by the results of our POMS' V score shown in Fig. 2, which shows that the vigor-activity scores after each mental stress task declined significantly compared with baseline scores; at the same time, with the GABA sample intake treatment, a smaller decrease in V scores after the stress load was shown. These results suggested that the oral intake of GABA might improve mood and partly prevent the decrease of brain activities that occurs after the mental stress load tasks used in this study.

Very interestingly, Fumoto et al. (2005) examined the effect of the oral reading of a classic text (Dokyo; Buddhist scriptures) on EEG, and found that the spectral power in the high-frequency alpha (HF-alpha) band (10–13 Hz) and beta band (13–30 Hz) increased 5 min after Dokyo and that the higher level was maintained thereafter. In addition, their subjects had a feeling of vigor-activity with reduced anxiety after Dokyo, as assessed by POMS scores. Moreover, the urinary and/or blood 5-hydroxytryptamine (5-HT) levels increased after Dokyo. Thus, they suggested that the increase in EEG activity is linked to the state of vigor-activity with reduced anxiety and that the serotonergic neurons within the brain may produce changes in the EEG patterns. A similar interpretation can be made of our results. The mental stress load tasks seemed to have a reverse effect compared with Dokyo, as they reduced vigor-activity and led to a decrease in EEG activity. It is known that the administration of GABA increases the concentrations of plasma growth hormone and the rate of protein synthesis in the brain (Tujioka et al. 2007, 2009), and this might have somehow influenced the brain wave activities and diminished the changes in the EEG patterns caused by stress load tasks.

The mechanism of how the oral intake of GABA affects the neurons within our brain and leads to its anti-stress function is still not clear. Further brain studies are expected to confirm this effect.

## Conclusion

EEG activities, including alpha band and beta band brain waves, decreased depending on the time course of the mental stress task loads, and at 30 min after GABA intake this decrease was diminished compared with the placebo condition. Thus, GABA might have alleviated the stress induced by the mental tasks. This effect also corresponded with the results of the POMS' V scores. The POMS' results also indicated that the mood-improving effect might last even longer, up to 90 min after the GABA intake.

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