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# Relaxation and immunity enhancement effects of γ-Aminobutyric acid (GABA) administration in humans

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**Abstract.** The effect of orally administrated γ-Aminobutyric acid (GABA) on relaxation and immunity during stress has been investigated in humans. Two studies were conducted. The first evaluated the effect of GABA intake by 13 subjects on their brain waves. Electroencephalograms (EEG) were obtained after 3 tests on each volunteer as follows: intake only water, GABA, or L-theanine. After 60 minutes of administration, GABA significantly increases alpha waves and decreases beta waves compared to water or L-theanine. These findings denote that GABA not only induces relaxation but also reduces anxiety. The second study was conducted to see the role of relaxant and anxiolytic effects of GABA intake on immunity in stressed volunteers. Eight acrophobic subjects were divided into 2 groups (placebo and GABA). All subjects were crossing a suspended bridge as a stressful stimulus. Immunoglobulin A (IgA) levels in their saliva were monitored during bridge crossing. Placebo group showed marked decrease of their IgA levels, while GABA group showed significantly higher levels. In conclusion, GABA could work effectively as a natural relaxant and its effects could be seen within 1 hour of its administration to induce relaxation and diminish anxiety. Moreover, GABA administration could enhance immunity under stress conditions.

Keywords: γ-Aminobutyric acid, brain waves, relaxation, stress, immunity, IgA

## 1. Introduction

γ–Aminobutyric acid (GABA) exists naturally in many kinds of foods at low levels while higher levels could be found in fermented food products. Therefore, production of natural GABA in high concentration could be produced by certain kinds of lactic acid bacteria [13]. Recently, GABA is used as a functional food ingredient in different kinds of foods in Japan due to its health benefits.

GABA is one of the major inhibitory neurotransmitters in the central nervous system and has been found in several peripheral tissues [3,12]. It is known to mediate pre-synaptic inhibition of primary afferent fibers in the motor system and may also be involved in post-synaptic forms of motor neuron inhibition [1]. Amino acid neurotransmitters are critical for the function of the central nervous system

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(CNS); they have fast actions, producing responses within few milliseconds playing an important role in brain functions and neurological diseases [20].

Moreover, GABA has a physiological role in many systems outside the CNS, including regulation cardiovascular functions [5,6,24,28], inhibition metastasis of cancer cells [16,21], regulation of pituitary functions and growth hormone production in rats [4,22], mammalian fertilization [10], and modulation renal function [12]. Moreover, it is well-proved that GABA or GABA-enhancing compounds, like the amino acid L-theanin, could play an important role in stabilizing mood disorders [14].

Electroencephalogram (EEG) is used to measure the brain waves; each wave pattern is associated with different mood state and state of consciousness. Alpha is one of four basic brain waves (delta, theta, alpha, and beta) which make up the EEG. Alpha is seen in wakefulness where there is a relaxed and effortless alertness. Beta is seen in highly stressful situations, and where there is difficult mental concentration and focus. Therefore, high alpha and less beta waves have been used as indices of relaxation, arousal, anti-stress, and better concentration [18,27].

Anxiety and stress are well-known to reduce the strength of our immune systems. Thus persons having more alpha and less beta waves could mean less anxiety and, correspondingly, stronger immune systems. Measuring of immunoglobulin A (IgA) in saliva would be a convenient and easy tool to monitor human immunity status [23]. It has been reported that IgA play an important role in protecting the mucosa of the upper respiratory tract and oral cavity against viral infection and bacterial adherence [25]. On the other hand, persons who have high neuroticism and anxiety are characterized by lower basal levels of IgA [7,9]. Furthermore, several studies demonstrated that relaxation leads to highly significant increase in saliva IgA concentrations. In other words, measuring IgA in saliva would be used as an indication of both immunity and mood states in human [2,8,26].

Under stress conditions certain metabolites, like GABA, are depleted, therefore, additional nutrients are required to replace these metabolites. As the quantities of nutrition vary from one individual to another and the difficulties to obtain these nutrients from food so a better alternative is to consume the required additional nutrients as enriched functional food and/or food supplements.

The aim of the present work was to investigate effects of orally administrated GABA on alpha and beta brain waves in healthy subjects as well as measuring IgA level in saliva of stressed humans.

## 2. Material and methods

### *2.1. Study I: Brain wave test*

Thirteen healthy Japanese volunteers, 7 males and 6 females, aged from 21 to 35 years, gave informed consent for participation in the present study. No subject had a history of behavioral disturbance, abuse of drug or alcohol, diabetes, or other pre-existing medical condition. Their bodyweight, height, and build were all within the normal range. Two hours before the study they were forbidden to eat, drink, or use any form of tobacco.

The study was approved and done using a multipurpose EEG, Telemeter (NEC Corp., Japan) at the Food and Nutritional Sciences Department, University of Shizuoka. The EEG was recorded before and after each of three administrations. The order of testing for each volunteer administered 200 ml of distilled water was as follows: (i) only distilled water; (ii) containing 100 mg of GABA produced by natural fermentation using specific strain of lactic acid bacteria (Pharma-GABA®, Pharma Foods International Co., Japan); and (iii) containing 200 mg L-theanine. Tests of the 3 administrations were separated by 7-day intervals. Electroencephalogram recordings were obtained with the subject resting

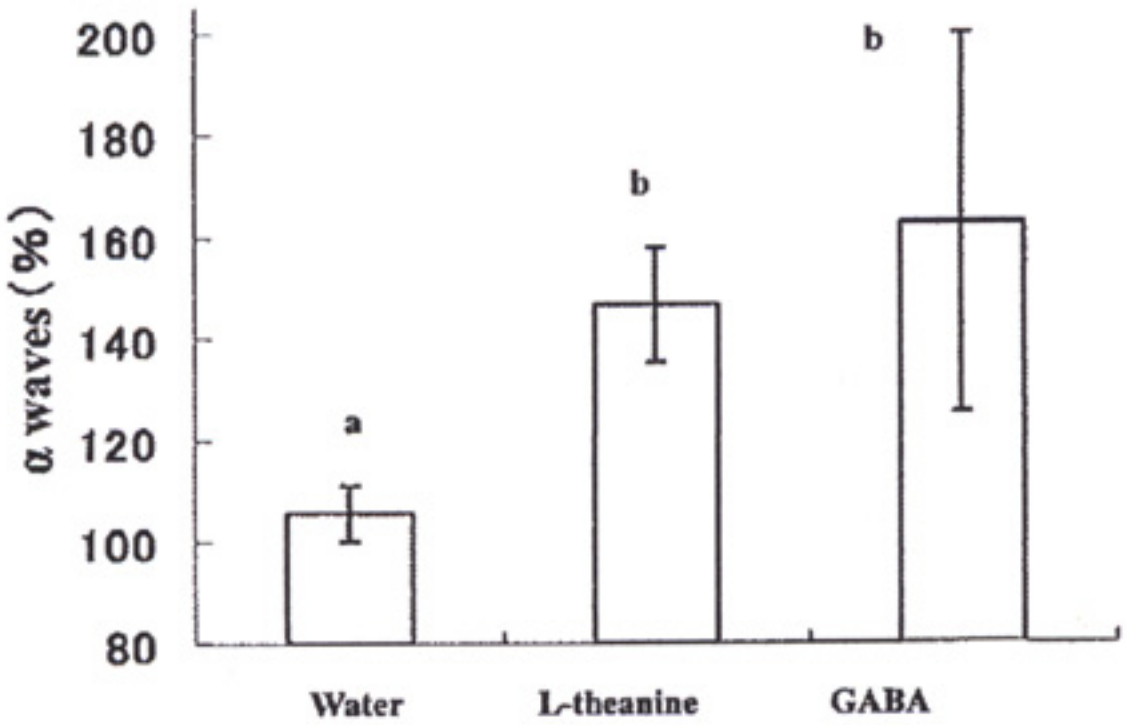


Fig. 1. Changes of alpha waves generation ratios after administration of water (control), L-theanine, and γ-aminobutyric acid (GABA) measured by electroencephalogram (EEG). Values are means ± SEM of waves ratios of 3 measurements (at 0, 30, and 60 minutes after each administration). Values with different letters are significantly different at $P < 0.05$.

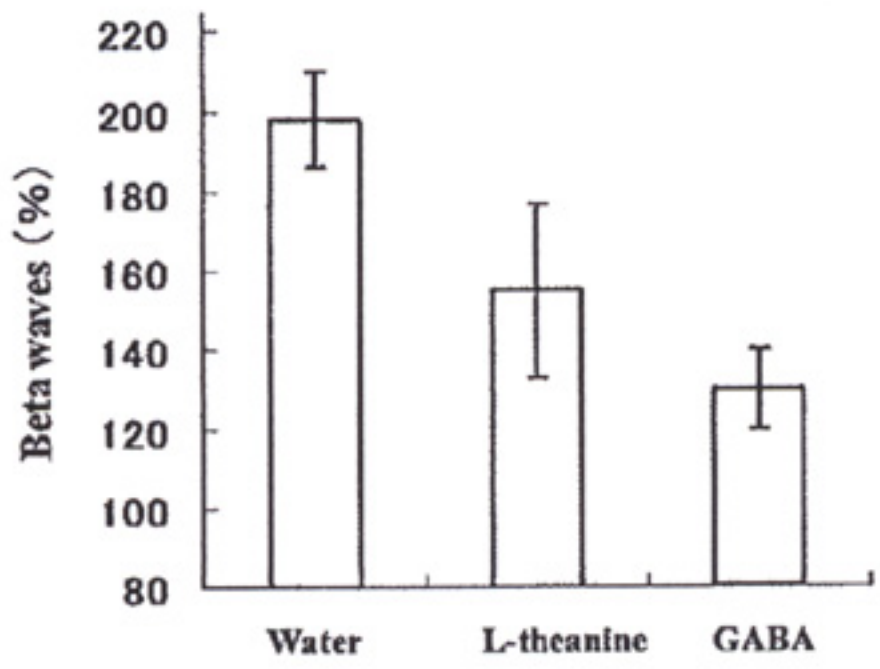


Fig. 2. Changes of beta waves generation ratios after administration of water (control), L-theanine, and γ-aminobutyric acid (GABA) measured by electroencephalogram (EEG). Values are means ± SEM of waves ratios of 3 measurements (at 0, 30, and 60 minutes after each administration).

quietly with closed eyes. Brain waves data were collected as follows: before administration, then at 0, 30, and 60 minutes after each administration for 5 minutes recording sessions. Alpha and beta waves were calculated as a percentage between pre and post-administration values. Alpha/beta ratios have been calculated as a ratio between alpha and beta percentage values.

### *2.2. Study II: Suspended bridge test (salivary IgA level monitoring)*

Eight healthy volunteers, with no clinical evidence of any illness, (5 males and 3 females) aged 25 to 30 years who had a history of acrophobia, were recruited. Immunoglobulin A level in subject's saliva was considered as a marker of relaxation, stress, and immunity response. Subjects crossed, as a tool of stress, a pedestrian suspended bridge at Nara Prefecture, Japan (Totsu River Bridge) with 54 m height, 300 m length, and 2 m width. Each participant was randomly assigned an identification number and assigned to a placebo or an experimental group. The participants had no knowledge of their group assignments.

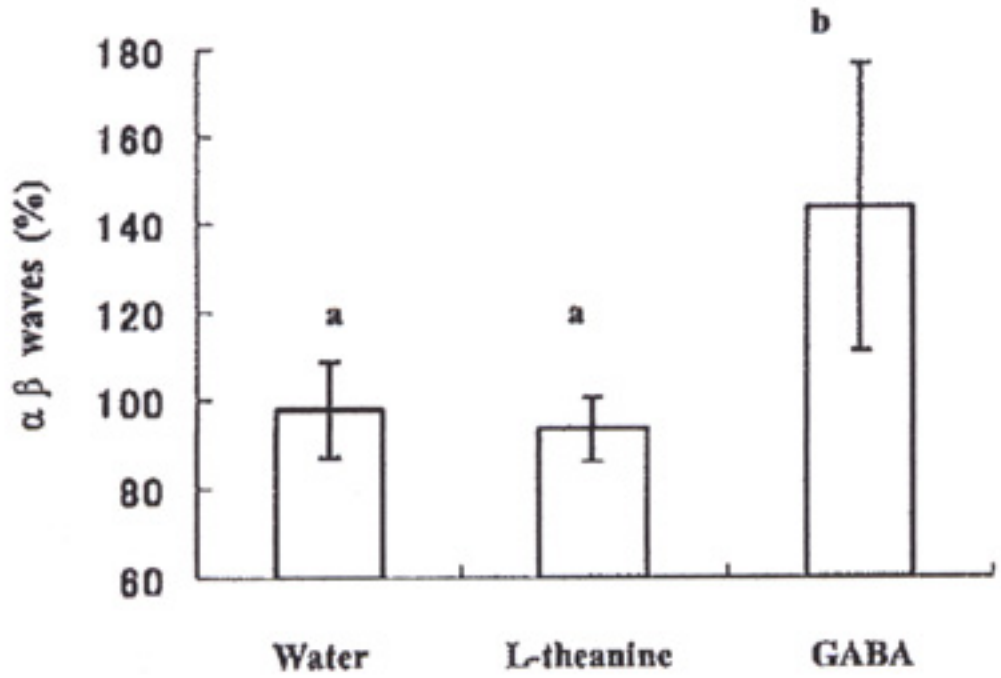


Fig. 3. Changes of alpha/beta waves ratio values after administration of water (control), L-theanine, and γ-aminobutyric acid (GABA) measured by electroencephalogram (EEG). Values are means ± SEM of alpha/beta ratio values of 3 measurements (at 0, 30, and 60 minutes after each administration). Values with different letters are significantly different at $P < 0.05$.

Saliva was sampled and each volunteer was chewing 3 cotton rolls for 1–2 minutes to assist saliva flow at 3 points; (i) before crossing (ii) at middle, and (iii) at end of the bridge. After being soaked with saliva, each roll was placed in a container that is closed with a plastic stopper, centrifuged, and then stored at −20°C until assayed. Some precautions have been taken to reduce the factors that affect saliva sampling: subjects should not eaten within 60 minutes prior sample collection, avoid alcohol intake for 24 hours, with good oral health, and avoid teeth brushing or oral abrasion. The concentration of IgA levels were determined by Human IgA enzyme-linked immunosorbent assay (ELISA) quantitation kit (Bethyl Laboratories, Inc., USA) as previously described [9].

### *2.3. Statistical analysis*

Data are presented as means ± SEM. Statistical analysis was performed with the unpaired student's t-test. Differences were considered statistically significant at $P < 0.05$.

## 3. Results and discussion

### *3.1. Study I: Changes in alpha and beta brain waves*

Amino acid neurotransmitters are critical for the central nervous system functions and can influence virtually every central neuron through activation of variety of receptors. It has been reviewed that amino acid neurotransmitter system dysfunction may contribute to the pathophysiology of mood disorders, and that correction of this dysfunction could be an important treatment tool for these disorders [14]. It is well known that GABA is one of the most important inhibitory neurotransmitters that ubiquitous in the CNS and peripheral tissues of mammals [3,19]. It has been suggested that plasma and cerebrospinal fluid GABA levels were reduced in patients with mood-disorders and similarly postmortem studies suggest that mood disorders are associated with reduced GABA levels in the cortical regions. We have carried out an animal study and data have shown that oral administration of GABA could result in an anxiolytic effects in rats (unpublished data), these findings open up several exciting possibilities for further exploration of the anti-stress effects that may be achieved by oral GABA intake in humans.

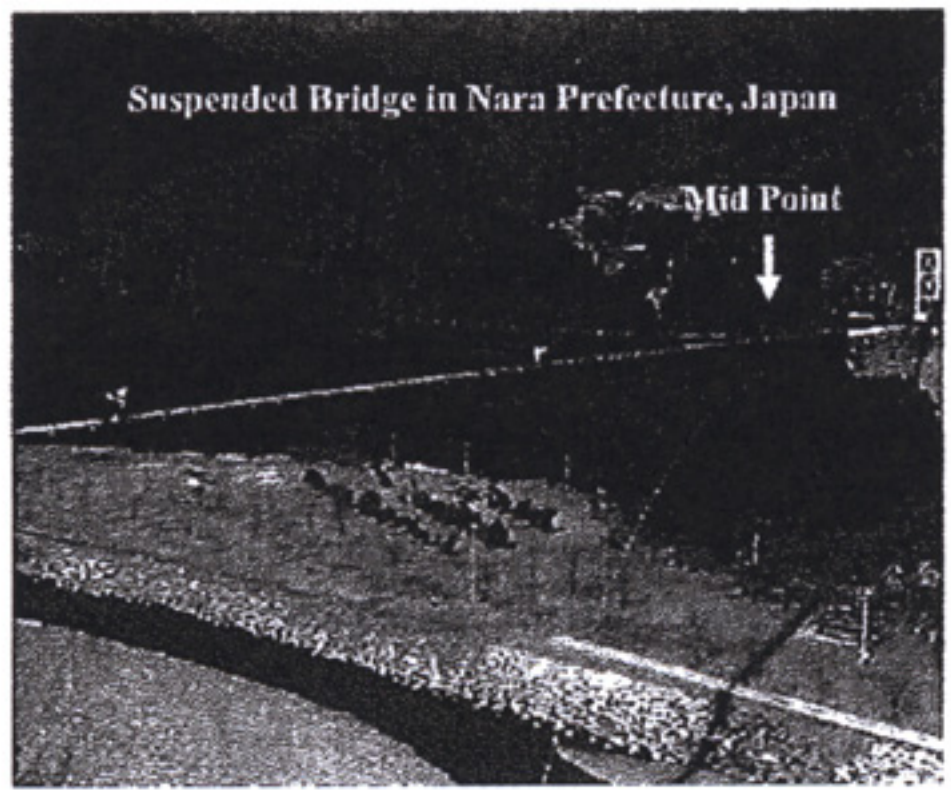


Fig. 4. Pedestrian suspended bridge (Nara Totsu River Village) with 300 m length, 54 m height, and 2 m width. Acrophobic volunteers are subjected to cross the bridge as a stress stimulus.

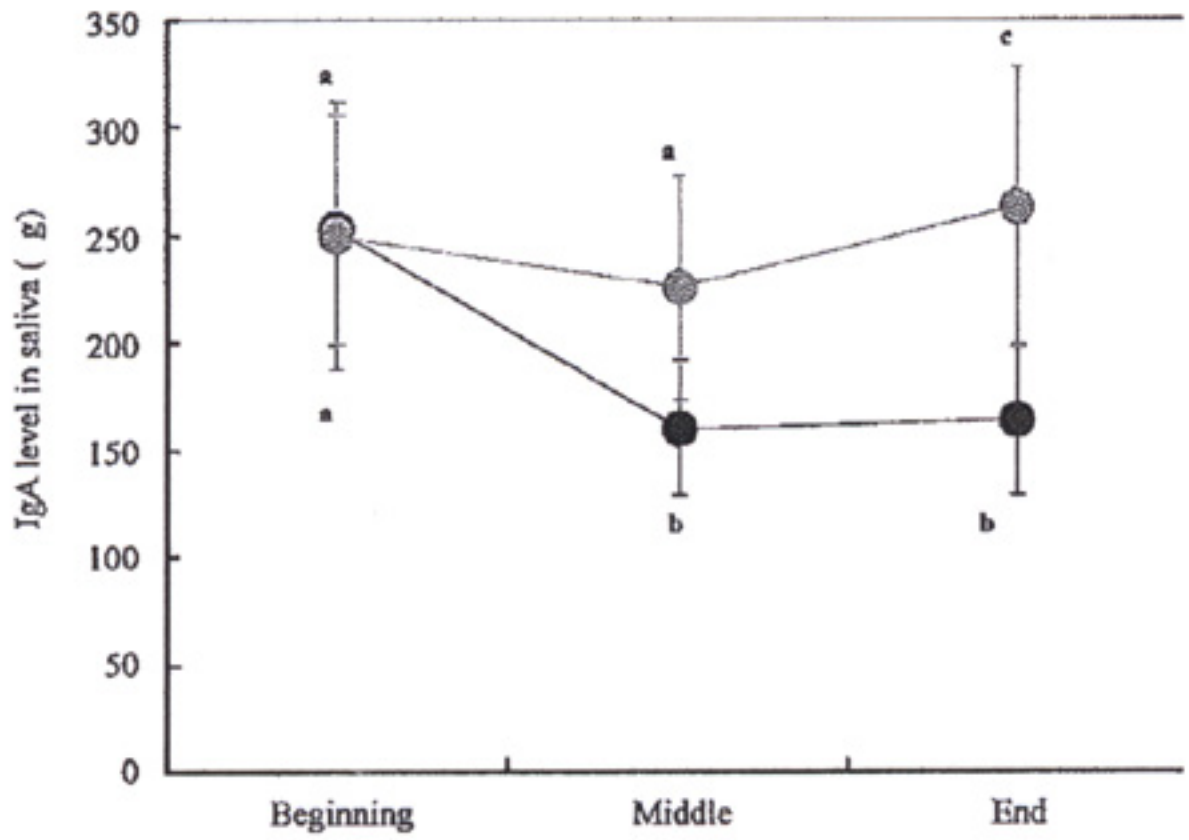


Fig. 5. Immunoglobulin A (IgA) levels in saliva of acrophobic volunteers crossing the suspended bridge as a tool of stress. Values are means ± SEM of IgA levels in 8 volunteers at beginning, middle and end of the bridge alpha/beta ratio values of 3 measurements. Values with different letters are significantly different at $P < 0.05$.

Electroencephalogram (EEG) detects electrical changes in the extracellular fluid of the brain in response to changes in potential among large groups of neurons. The intensity of the electrical changes is directly related to the degree of neuronal activity. Brain waves are classified according to their frequency into four types: alpha (less than 8–13 Hz), beta (more than 13 Hz), theta (less than 4–8 Hz), and delta waves (less than 4 Hz). Each wave's type is associated with a certain mental status. Delta and theta occur during deep sleep and early stages of sleep, respectively. Alpha is generated in relaxed and effortless alertness, while beta is seen in highly stressful situation and where there is a difficulty in mental concentration. Therefore, alpha and beta waves are used as indices of arousal, relax, concentration, stress and anxiety conditions in human [18,27]. Brain waves data were collected at 0, 30, and 60 minutes based on GABA

concentration profile in blood after oral administration of GABA. In our previous animal study; Wistar male rats orally administered GABA (100 mg/100 g body weight), and GABA concentration in their blood showed its peak after 30 minutes, maintains its high concentration up to 60 minutes and then started to declines. The experiment was repeated after continuous feeding of GABA for 11 days and the results showed that GABA concentration profile in their blood was almost the same (unpublished data). Therefore, measuring of brain waves was selected to be measured whereas GABA has high blood concentration.

Based on the results of the present study, administration of 100 mg GABA led to a significant increase in alpha waves compared to control (Fig. 1). Meanwhile, a marked decrease in beta waves has been found with the same dose of GABA (Fig. 2). These results would suggest that GABA intake has relaxing and anxiolytic effects. In our preliminary studies, we have found that 200 mg GABA intake showed marked increase in alpha and decrease in beta waves so 100 mg have been planned in the present study (unpublished data).

Previous studies have demonstrated an association between alpha waves and the psychosomatic state as reflected on the EEG [18,27]. Therefore, the changes in EEG in the present study that were seen after GABA administration suggested a relaxation status with respect to psychosomatic status as compared with water administration. To our knowledge, this is the first report to demonstrate that GABA administration significantly induces relaxation and reduce anxiety in humans.

On the other hand, L-theanine is an amino acid found primarily in green tea and historically has been shown to have relaxing properties. Recently, it has been reported that L-theanine has the ability to increase alpha waves in the brain [11,15]. It has been reported that the recommended dose of L-theanine is 200 mg, which showed some evidence for a relaxing effect under resting conditions; however, the same dose of L-theanin had no significant anxiolytic effect in healthy subjects as determined by behavioral measures of anxiety [15].

In agreement, we found that L-theanine intake resulted in an increase in alpha waves, however, it was comparatively less than that generated by GABA administration (Fig. 1). Meanwhile, L-theanine intake showed less decrease in beta waves compared to GABA intake (Fig. 2). Furthermore, a ratio between alpha/beta waves has been calculated to indicate the harmonious status of relaxation and anti-stress conditions [18,27]. A significant high alpha/beta ratio values were obtained after GABA administration compared to both water and L-theanine administrations (Fig. 3), that would be interpreted as reflecting a status of arousal and extreme relaxation, the so called relaxed concentration [17].

The results obtained from changes in brain waves detected by EEG showed that administration of GABA not only could induce an arousal status with relaxation but also could reduce the status of stress and anxiety.

### *3.2. Study II: Suspended Bridge test (salivary IgA level monitoring)*

Immunoglobulin A (IgA) in saliva has been suggested to play an important role in protecting the mucosa of the upper respiratory tract and the oral cavity against viral infections and bacterial adherence [25]. Subjects high in anxiety are characterized by lower basal levels of IgA and by more pronounced decrease in IgA in certain stress conditions [7,9]. Several studies have demonstrated that relaxation consistently leads to highly significant increases in IgA concentrations [2,8,23,26]. Measuring of IgA in saliva is considered as an easy non-invasive and stress-free nature of collection. Therefore, IgA level in saliva could be used as an index of stress and/or relaxation status in humans.

Saliva as a diagnostic fluid may be useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases, and endocrine disorders. However, some factors may affect

the obtained results, so special precautions including sampling time, quantity, oral health and diet should be taken. To overcome the variation in saliva quantity between subjects; the absolute concentration (μg/ml) of salivary IgA was determined by regression analysis. The output of IgA (μg/min) was calculated by multiplying the absolute concentration by salivary flow rate (ml/min).

Our preliminary study of the effect of oral GABA intake on salivary IgA level in 13 volunteers, in rest conditions, showed that a significant increase in IgA level after 90 minutes of GABA intake (unpublished data). Therefore, the effect of GABA administration on immunity of stressed human has been carried out in this study. Volunteers with a history of acrophobia have been subjected to stress by crossing a suspended bridge (Nara Totsu River Village). The bridge is known as the Japan's longest pedestrian suspension bridge with a length of 300 m, height 54 m, and width 2 m (Fig. 4). A markedly decrease of IgA levels were found in saliva of placebo group at the middle and end of the bridge, while in GABA group, IgA levels were changed from slightly lower levels at the middle to significantly higher levels at the bridge end (Fig. 5). In this experiment, the results denoted that GABA administration not only interferes with lowering IgA but increase its levels even in stressful conditions.

Anxiety and stress have been implicated in heart disease, hypertension, ulcers, depression, decrease immunity and other diseases [14]. Moreover, it has been estimated that over 75% of all disease prevalence is related to the activation of the stress mechanism, and that more than 66% of all visits to primary-care physicians are for stress related disorders. While the causes of anxiety and stress vary individually, the changes which take place in the body being under stress are similar. It has been recorded that stress depletes neuroregulators from the endogenous opioid, GABA, and serotonin systems. On the other hand, stress increases the dopamine and norepinephrine release. Such neuroregulator changes produce a combination anxiety and alertness [14]. Therefore, it is suggested that the relaxation and anti-anxiety effects of GABA intake, as shown in study I, would be the reason of elevating IgA levels. Thus GABA intake can contribute to enhance human immunity even under stressful conditions.

## 4. Conclusion

Oral administration of this natural relaxant amino acid, GABA, that is produced by natural fermentation of specific strain of lactic acid bacteria works effectively within 1 hour to diminish stress, worry, anxiety, and may allow the brain to focus and concentrate better. Moreover, even in stressful conditions, GABA administration could elevate the human immunity through its relaxant and anxiolytic effects.

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