Memory is the process of acquiring, storing, and retrieving information. Learning is the skill of acquiring and encoding information about an object and its context. This skill is essential and allows for the effective retrieval of information. The length of time that a behavior continues determines its degree of retention in short term memory (minutes to hours) and long term memory (days to years) (1). Recognition memory is the ability to recognize a previously experienced item (2). It requires a learning process in which an object is identified and information about its environment is perceived (3, 4). Moreover, investigators have indicated that different types of memory formation require a variety of memory systems in different areas of the brain (3, 4).

Working memory can be defined as the systems that are essential for keeping information in mind while performing complex tasks, e.g. reasoning, comprehension, and learning (5). Many studies have shown that γ-aminobutyric acid (GABA) is involved in working memory processes (6, 7). GABA depletion in the prefrontal cortex causes deficits in delay-task performance in the monkey (7). Fuster (8) demonstrated that GABA-ergic interneurons are importantly involved in the encoding and maintenance of information in working memory. However, the effect of GABA intake on the working memory is still unclear.

GABA is found naturally in many foods, such as tomatoes, unpolished rice, chocolate, tea, and other foods. It is an important inhibitory neurotransmitter. However, the influence of GABA on object recognition and working memory is still unknown. In this study, the effects of GABA on novel object recognition (NOR) memory and working memory were examined. The proper retention interval and delay time were also investigated for the NOR test and T-maze test, respectively. Male 3-wk-old Wistar rats were allowed free access to food and water containing 0.5% GABA or 1% GABA for a month. After that, the rats performed the NOR test at a 48 h retention interval and T-maze test at a 900 s delay time to estimate the effects of GABA on learning behavior. The results showed that the object information in the NOR test was stored as long-term memory and the recognition index (RI) was significantly increased after GABA administration. The accuracy rate also significantly increased after GABA administration. These indicate that GABA may be involved in long-term object recognition memory and working memory.

Key Words  GABA, novel object recognition, T-maze, working memory, learning behavior

Note

GABA Affects Novel Object Recognition Memory and Working Memory in Rats

Panicha THANAPREETAWAT1, Hiroki KOBAYASHI1, Naoto INUI1, Kazuhiro SAKAMOTO1, Mujo KIM2, Ai YOTO1 and Hidehiko YOKOGOSHI1,4,*

1Laboratory of Nutritional Biochemistry and Global COE Program, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka 422–8526, Japan
2Laboratory of Research Department 3, Pharma Foods International Company Ltd., Kyoto 615–8245, Japan
3Laboratory of Nutritional Biochemistry, School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka 422–8526, Japan
4Laboratory of Nutritional Biochemistry, College of Bioscience and Biotechnology, Chubu University, Aichi 487–8501, Japan

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Summary γ-Aminobutyric acid (GABA) is an amino acid found in unpolished rice, chocolate, tea, and other foods. It is an important inhibitory neurotransmitter. However, the influence of GABA on object recognition and working memory is still unknown. In this study, the effects of GABA on novel object recognition (NOR) memory and working memory were examined. The proper retention interval and delay time were also investigated for the NOR test and T-maze test, respectively. Male 3-wk-old Wistar rats were allowed free access to food and water containing 0.5% GABA or 1% GABA for a month. After that, the rats performed the NOR test at a 48 h retention interval and T-maze test at a 900 s delay time to estimate the effects of GABA on learning behavior. The results showed that the object information in the NOR test was stored as long-term memory and the recognition index (RI) was significantly increased after GABA administration. The accuracy rate also significantly increased after GABA administration. These indicate that GABA may be involved in long-term object recognition memory and working memory.

Key Words  GABA, novel object recognition, T-maze, working memory, learning behavior

Abbreviations: GABA, γ-aminobutyric acid; NOR, novel object recognition; RI, recognition index.

*To whom correspondence should be addressed.
E-mail: yokogoshi@isc.chubu.ac.jp
familiar arm. Jeneson and Squire (11) suggested performance on tasks involving long delays frequently requires long-term memory where attention is diverted, while working memory is sufficient for the tasks involving short delays and the amount of information to be maintained is limited. In the present study, the NOR test was used to evaluate the long-term object recognition memory. However, the NOR test is not appropriate to determine the influence of GABA on working memory as the retention interval was too long and the amount of information to be maintained is overwhelming for testing working memory. Therefore, the delayed non-matching-to-sample (DNMTS) task in a T-maze test is a suitable paradigm to assess the performance dependent on working memory.

Materials and Methods

Animals and diets. Male 3-wk-old Wistar rats (Japan SLC, Inc., Hamamatsu, Japan) weighing approximately 60 g were housed at 24±1°C, 55±5% humidity in a room with a 12-h light/dark cycle. The rats were housed 4–5 rats per cage. Experiments in this study were carried out in accordance with Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka, which are based on the American Association for Laboratory Animals Science standards.

Retention intervals. In this experiment, rats were divided into 3 groups, i.e. control–1 h, control–24 h, and control–48 h. Rats (n=5) received food (standard diet, CE-2 CLEA Japan, Inc., Tokyo) and tap water. On the day of the experiment (the sample trial, T1), rats received the diets 30 min prior to the test. Then rats were habituated to the empty chamber before two identical objects were placed centrally in the chamber (9 cm from the walls); the rats were allowed to explore the chamber and the objects (A1 and A2) for 5 min. In the second trial (the choice trial, T2), the encountered object (A2) was replaced with a novel object (B2). The objects and the ground of the box were wiped with a piece of cloth, which was saturated in the natural odor of rats to prevent the rats from receiving olfactory cues from scent traces left on the objects. Then the rats were allowed to explore and observe the objects for 3 min. The retention intervals between T1 and T2 were 1, 24 and 48 h. Exploration was considered to have occurred when the rats faced the object at a distance ≤2 cm and/or touched it with their noses. Circling the object or sitting on it were not considered exploration. The performance of the rats was monitored on a closed-circuit TV screen in the next room, and their exploration time was manually recorded. All objects used in the device and the relative positions of objects were counterbalanced and randomly permuted.

Evaluation of the rats’ recognition memory was expressed as percentage of the recognition index (RI), according to the following formula.

\[ RI(%) = \left( \frac{N}{N+F} \right) \times 100 \]

where N = the time spent exploring the novel object (B2), and F = the time spent exploring the familiar object (A1).

After the completion of the NOR test, the rats were trained on the T-maze test.

Delay times. After the retention interval test, rats (n=5) performed the delay-time T-maze test. The test followed previous studies by Le Greves et al. (12), Liu and Collie (13), Furukawa et al. (14), and Grzeda et al. (15). Briefly, the test was performed using a T-shaped maze made of wood painted black inside. The size of the start arm was 59 cm×9 cm×15 cm, and the two goal arms were 50 cm×9 cm×15 cm. The upper surface of the maze was capped with an acrylic plate. A piece of standard laboratory chow was placed on a 4 cm diameter pottery container. Rats were deprived of food for 24 h before the learning session. For the first 3 d of the training session, 3 g of food was placed in both goal arms, and then the rats were allowed to freely explore and familiarize themselves with the maze for 10 min. On day 4 of the training session, the learning phase was conducted by closing one of the goal arms with a sliding door (guillotine) to train the rats to enter the open goal arm. Food was provided at the end of the open goal arm and the rat was removed from the maze after eating. The location of the open arm was counterbalanced. The maze was carefully cleaned after the learning session. Each rat was trained 4 times with a 4 min interval after each training session. On day 5 to 8 of the training session, the test phase was conducted with both goal arms opened, but only the previously closed arm contained food. Rats tended to explore the previously closed arm. Each rat was given 4 training sessions per day, with a 4 min interval after each training session. Rats which either did not start moving into the maze within 1 min or had an accuracy rate of less than 75% were excluded from the test. A delay period of 3, 300, 900, and 1,800 s was introduced between the learning and test phases. The rats were kept in the cage during the delay time. Then a rat was immediately placed in the start arm. The test was repeated 5 times over 2 d. The percentage of accuracy rate was calculated for each animal.

Open field test and the effect of GABA on NOR memory. In this experiment, rats were divided into 3 groups, i.e. control, 0.5% GABA, and 1% GABA. The rats performing in this experiment were not the same rats tested in the retention interval experiment. The control group (n=8) received food and water without GABA, and the other two groups (n=8) received food and water containing 0.5% or 1% GABA. Water intake and body weight were measured daily. After 1 mo, the rats performed the open field and NOR test to estimate the effect of GABA. The procedure was partially modified from the method of Puma et al. (16) and Bertaina-Anglade et al. (17). An open field test was conducted on the first and second days of the experiment. The apparatus, which was an open square box (70×70×40 cm) made of plastic walls and a black plastic floor, was placed in a dark room under infrared rays, because of videotaped during an open field test and replayed for analysis. The rats were allowed to explore the empty open field for 5 min. The rats received the diets 30 min prior to performing the tests. The procedure of the NOR test repeated the
retention interval experiment with retention intervals.

**T-maze test.** The same rats (n=8) from the NOR test were used in the T-maze behavioral test with a delay time of 900 s. The procedure of this experiment was the same as the delay time experiment, which followed Le Greves et al. (12), Liu and Collie (13), Furukawa et al. (14), and Grzeda et al. (15).

**Statistical analysis.** Data are given as means±SE. Statistical analysis was performed with the one-way ANOVA t-test to determine whether there were significant (α=0.05) differences among the groups, followed by the post-hoc Tukey-Kramer and Games-Howell tests to determine which means were significantly different. A p value of less than 0.05 was considered significant.

**Results and Discussion**

Previous studies have already shown that GABA is involved in memory formation. This study reveals the behavioral effects of GABA administered in water. The body weight of the rats in all three groups increased gradually as they grew older. However, there was no significant difference in the body weight among the three groups (Fig. 1). The amount of GABA administered was approximately 0 mg (control), 119 mg (0.5% GABA), and 238 mg (1% GABA). The animals’ performance in the open field is shown in Table 1. The total locomotion (cm) (F(2,21)=0.874, p>0.05) and % center (F(2,21)=0.313, p<0.05) of the rats was not significantly different among the three groups. Therefore, GABA neither affects locomotor activity, level of anxiety nor body weight.

The effect of the retention interval on NOR memory was studied in order to determine the retention time for the NOR test. Rats in the control groups performed the NOR task with retention times of 1, 24, or 48 h. The results showed a significant decrease in the RI of the control at 48 h (52±3%) compared to 1 h (67±3%) (F(2,12)=4.554, p<0.05). In addition, retention intervals did not affect the exploratory activity. Hence, 48 h was selected as the retention time for the NOR test to study the effect of GABA on NOR memory.

Table 2 shows the time spent exploring the object A1 in T1 of all groups was around 20–30 s. However, there was no significant difference among the three groups (F(2,21)=1.246, p>0.05). In T2, all rats spent a shorter time exploring the familiar object compared to
the exploration time in T1 (Table 2). However, the time spent exploring the familiar object (T2) by the 1% GABA group was significantly shorter than that of the control \((F_{(2,21)} = 3.585, p<0.05)\), while there was no significant difference between control and 0.5% GABA \((p>0.05)\). Table 3 shows the RI of the control group, 0.5% GABA, and 1% GABA. GABA intake resulted in a significant increase in RI compared to the control group \((p<0.05)\). However, there was no significant difference in the RI between the 0.5% and 1% GABA groups \((p<0.05)\).

A number of investigators have suggested that several brain regions are involved in the recognition process. Brown and Aggleton (3) and Yonelinas et al. (4) proposed that the recognition process consists of at least two processes, i.e. recognition of familiar things, and recall of contextual information related to those things. Hence, recognition of a part of a contextual clue could lead to remembering the familiar object in the NOR test, and to exploring the new arm in the T-maze task. In the present study, the increase in the recognition index after GABA was administered indicates that GABA affected the recognition memory of the rats. However, there were very few contextual habituations in this study, e.g. the room used in the NOR test was dark and no olfactory cues existed. The time spent exploring the familiar object in T1 of all groups was around 20–30 s, which was long enough for the rats to encode the object’s information. Moreover, the exploration time of all groups for the familiar object in T2 was less than both the exploration time for the encountered object in the sample phase and the time spent exploring the novel object. In addition, there was a repetition of the learning and test sessions, and the retention interval of the NOR test appears to have been long enough for the consolidation process. Therefore, in this case, it is very likely that the object’s information was stored in the brain as long-term memory. Nevertheless, in the present study, GABA concentration was not related to an increase in the RI.

Previous studies have shown that protein synthesis in the brain is important for the memory formation process (18–20). Investigators have reported that growth hormone was increased after GABA intake (21–23). Moreover, previous studies have shown that the increase in growth hormone level correlated to protein synthesis rate in the brain (21). In addition, several investigators have demonstrated that memory function of adult patients with growth hormone deficiency was improved after growth hormone treatment (24, 25). Therefore, GABA may affect protein synthesis in those brain areas relating to object recognition memory. However, further studies are required to confirm this hypothesis.

The accuracy rate was significantly decreased at 900 s (18±4%) and 1,800 s (6±2%) compared to 3 s (82±6%) \((F_{(3,16)} = 51.256, p<0.05)\). In other words, the working memory of the rats had almost completely faded at the 900 s delay time. Thus, the DNMTS was conducted at a delay time of 900 s. Table 3 shows an increase in percent accuracy rate after GABA intake. The accuracy rate of the 0.5% GABA group was significantly higher than that of the control \((F_{(2,21)} = 6.573, p<0.05)\). In addition, although the accuracy rate increased after 1% GABA administration, it was not significantly different from that of the control and the 0.5% GABA groups \((p<0.05)\). Jeneson and Squire (11) suggested that the amount of information that could be kept in mind and the importance of the information were the indices to determine whether the performance relied only on working memory or also depended on long-term memory. However, long-term memory was required to support the performance when attention was diverted (11). In addition, attention tends to be diverted as the retention time after learning increases; a shorter retention interval decreases the chance that attention will be diverted (11). Therefore, in the present study, working memory appears to be sufficient for the performance in the T-maze task at the delay time of 900 s as the working memory had almost faded and 1,800 s may be too long to maintain information in working memory. As for the accuracy rate, the rate increased significantly after GABA intake. This indicates that the rats administered GABA could maintain information in mind for a longer time compared to the rats drinking only tap water. However, the accuracy rate was not correlated with an increase in the concentration of GABA. Stanton et al. (26) showed that posterodorsal septal lesions impair behavioral functions, resulting in a decrease in the accuracy rate in both the delayed matching to sample (DMTS) and delayed non-matching to sample (DNMTS) tests. Furthermore, declines in the accuracy rate in the DNMTS task in T-maze test have been reported in relation to lesions in the inside septum (27), outside septum (28), entorhinal cortex (29), and the CA3 region of the hippocampus (30, 31). Additionally, Baddeley et al. (32) suggested that the hippocampus is involved in working memory. Similarly, hippocampal, amygdala, and caudate nucleus lesions revealed that working memory requires a functioning hippocampus, while reference memory requires a functioning dorsal septum (17). Our study indicates that GABA might affect the hippocampus and/or caudate nucleus septum, which in turn affect the maintenance of working memory.

The present study has demonstrated that administration of GABA appears to be related to the maintenance of both long-term NOR memory and working memory. GABA might contribute to protein synthesis in the brain regions participating in the processes of learning and recognition memory by affecting the level of growth.

Table 3. The RI (%) at 48 h of retention interval and accuracy rate (%) at delay time of 900 s of rats in control, 0.5% GABA, and 1% GABA groups.1

| Recognition time (%) | Accuracy rate (%) |
|----------------------|-------------------|
| Control              | 53.8±1.7          | 54.2±3.7        |
| 0.5% GABA            | 60.8±1.2*         | 70.0±3.1*       |
| 1% GABA              | 66.5±2.5*         | 60.0±2.5        |

1 Values are means and SE, n=8. *Significant difference from the controls \((p<0.05)\).
hormone. Future studies will be concerned with evaluating the influence of hormones, neurotrophic factors, and protein synthesis in the regions of the brain related to recognition and working memory, e.g. the hippocampus and cortex following GABA intake on learning behavior and memory. Moreover, task difficulty, as well as stress and interference, are also important factors to be considered: thus, a variety of behavioral assessments should be carried out to clarify the role of GABA in working memory, reference memory, and learning behavior.

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