We identified an effect of theanine on memory functions in a novel object test. Rats were fed theanine for 3 weeks ad libitum, and then they performed the object test. The theanine-fed group performed search behavior for the novel object in the test session. The results suggest that theanine-fed rats showed improved recognition, and that theanine affected learning and memory.

Key words: theanine; amino acid; behavior; memory; novel object test

We found that theanine, a unique amino acid in tea leaves, has a significant effect on memory in a rat behavioral test. Rats were fed theanine for 3 weeks to see if it improved their ability to recognize new objects. The results show that rats fed theanine performed better in recognizing the new objects compared to the control group. Theanine is known to affect the nervous system, and this study suggests it could have potential for enhancing memory function.
During the retention test, the animal was placed back in the same box, in which one of the familiar objects used in training was replaced with a novel object, and was allowed to explore freely for 3 min. In test session 2, a novel object was replaced by another object. A preference index, the ratio of time spent exploring the original object (training session) or the novel object (retention session) to the total time spent exploring both objects, was used to measure recognition memory. Differences between the two groups were analyzed by Student’s t-test. In all cases, \( P < 0.05 \) was considered significant. The results are expressed as the mean ± SEM.

The novel-object recognition task is a simple test measuring visual-related memory. Tang et al. estimated learning and memory in glutamate-receptor genetically modified rats by the novel object test.

Transgenic mice did not differ from normal mice in point of the exploratory ratio of two objects in the training session. After a few days, in the retention test, transgenic mice remembered the original object and

**Fig. 1.** Effects of Theanine Consumption on Body Weights of Rats (A) and Water Intake (B).

Theanine-group rats drank tap water containing 2% theanine *ad libitum* for 3 weeks. Control rats drank tap water. Values are the means ± SEM (n = 8).
devoted their time to exploring the new one. There was no significant difference in the amount of time the rats spent exploring the two objects during the training session (Fig. 2A).

During the retention test, one of the familiar objects used in the training session was replaced with a third novel object, and the animals were allowed to explore for 3 min. Theanine-fed and the control group exhibited a similar preference towards the novel object in the 1-h retention test. This indicates that the two groups were equally able to retain a memory of the original object for 1 h, but when the retention test was conducted 2 d later, the theanine-fed group animals exhibited a much stronger preference for the novel object than the control group (Fig. 2B). This result suggests that the theanine-fed rats used long-term memory. The theanine-fed animals showed the same proportion of cognitive behavior as the control animals in this test, but the total exploration time of the two groups was different (Fig. 2C). That of the theanine-fed animals was shorter than that of control group animals in the training and retention sessions. A behavioral change, such as loco-

Fig. 2. Effects of Theanine on Exploratory Preference in the Training Session (A), the Test Session (B) and the Novel Object Test, and on Exploration Time (C).

Theanine-group rats drank tap water containing 2% theanine *ad libitum*. Control rats drank tap water. Exploratory preference shows the percentage of exploration time of one of two objects (training session), with respect to a novel object (test session). The total exploration time is shown as the time the rats spent exploring in the training session (5 min) and the test session (3 min). Values are the means ± SEM (n = 8).

*Significantly different from the control group (p < 0.05).
motor activity, influences the estimation of learning and memory in animal studies. Hence, we measured the locomotor activity in the rats by the open field test. The rats were kept same conditions as in novel-object test, and the open field test was performed. The open-field box size was (70 cm × 70 cm × 40 cm in height). The rat moved into the open field box for 5 min and its behavior was recorded. Theanine consumption did not change locomotor activity (Table 1). We suggest that this behavioral change due to theanine consumption involved a change in these emotion-relating neurotransmitters. We hypothesized that theanine consumption increased the fear or wariness of rats, but in an elevated plus-maze test, theanine consumption did not affect the fear-related behavior or locomotor activity of the rats (data not shown).

In conclusion, long-term theanine consumption might improve learning and memory, but the detailed mechanism is not clear yet, and further experiments are necessary to prove the effects of theanine on learning behavior. In addition, since other factors, such as emotional effects, cannot be fully excluded, further studies with other measurement methods for learning and memory are needed to confirm the ameliorating effects of theanine on learning and memory.

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<th>4 min</th>
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Table 1. Effects of Theanine on Locomotor Activity in the Rats

Theanine-group rats drank tap water containing 2% theanine ad libitum. Control rats drank tap water. The open field test was performed for 5 min. Values are the means ± SEM (n = 8).

References


