Chapter 61

Theanine and amelioration of brain stress

Keiko Unno
Tea Science Center, University of Shizuoka, Suruga-ku, Shizuoka, Japan

List of abbreviations

CE/TA ratio The molar ratio of the sum of caffeine (C) and EGCG (E) to the sum of theanine (T) and arginine (A)
Dnmt3a DNA methyltransferase
EGCG Epigallocatechin gallate
GABA γ-amino butyric acid
Holo-GR Agonist-bound glucocorticoid receptor
HPA Hypothalamus-pituitary-adrenal
IDO Indoleamine-2,3-dioxygenase
Kyn Kynurenine
Lcn2 Lipocalin 2
MRI Magnetic resonance imaging
Npas4 Neuronal PAS domain protein 4
REST Repressor element 1 silencing transcription factor
ROS Reactive oxygen species
SAMP10 Senescence-accelerated mouse prone 10
STAI State-trait anxiety inventory
VAS Visual analogue scale

Introduction

Theanine (L-theanine) is an amino acid found abundantly in green tea, in the form of ethylamine bound to glutamic acid (Fig. 61.1). It is a unique amino acid that is rarely found in plants, except in the tea plant. Theanine accounts for 1%−2% of the content of dried tea leaves and is especially abundant in high-grade green teas as one of its tasty components.

Since theanine is structurally similar to glutamic acid, a neurotransmitter in the brain, it is thought to have some physiological effects in the brain and has thus been the subject of much research. In studies using experimental animals, it was found that theanine is absorbed from the intestine and then transported into the brain via the blood−brain barrier (Terashima et al., 1999), affecting neurotransmitters such as dopamine (Yamada et al., 2009; Yokogoshi et al., 1998), and inhibiting the excitatory effects of caffeine (Kakuda et al., 2000), while studies using cultured cells reported its involvement in neuronal cell regeneration (Yoneda et al., 2019, 2020). In humans, theanine has been reported to relax the brain (Kobayashi et al., 1998), reduce stress (Kimura et al., 2007; Unno, Tanida et al., 2013), improve memory (Baba et al., 2021; Kakuda, 2011), and alleviate depression and schizophrenia (Hidese et al., 2017; Ota et al., 2015).

This chapter describes the stress-reducing effects of theanine. Moderate stress is considered to be necessary and has positive effects, whereas excessive and/or prolonged stress load may cause the onset or worsening of various diseases, including depression, mood disorders, cardiovascular diseases, and aging-related diseases (Juruena et al., 2020; Dar et al., 2019). It is well known that Alzheimer's disease causes brain atrophy, but repeated exposure to intense stress can also cause brain atrophy in healthy individuals (Ansell et al., 2012). Brain atrophy has also been reported in children who have been abused through violence, verbal abuse, or neglect (Fujisawa et al., 2018). Animal studies have been used to focus on changes in the brain associated with stress loading. In addition, we will introduce case studies in which theanine was shown to reduce stress in human clinical trials. The stress-reducing effects of green tea will be presented, including the effects of the major components of green tea, caffeine and catechins, on theanine.

Stress-relieving effects of theanine in animal studies

We investigated the stress-relieving effect of theanine using laboratory animals. Normally, mice are kept in groups of several mice per cage, but when two male mice are kept separate in a cage with a dividing board, they establish a sense of territoriality. Later, when the same two mice are

Tea in Health and Disease Prevention. https://doi.org/10.1016/B978-0-443-14158-4.00079-8

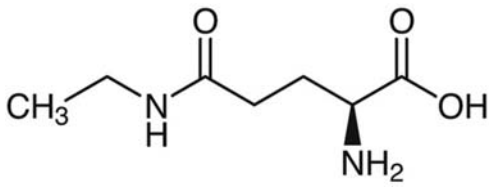

FIGURE 61.1 Structure of theanine.

reared without the dividing board, they become stressed by the presence of the other in their territory (Fig. 61.2). Although these mice appear to be living in harmony with each other, they are quite irritated because they fight easily over the slightest thing.

A situation of confrontational rearing causes stress in mice in the presence of other mice, and thus social psychological stress—similar to stress in humans—is also imposed on mice (Unno, Iguchi et al., 2013). When stress is applied to an organism, stress responses such as changes in hormone secretion and adrenal hypertrophy are observed due to activation of the HPA axis, which consists of the hypothalamus, pituitary gland, and adrenal cortex, via excitatory signals.

When mice of the outbred strain ddY, a strain commonly used in experiments, were used under confrontational rearing, the diurnal rhythm of glucocorticoids was altered and adrenal hypertrophy was observed, but these conditions were normalized in mice that ingested drinking water containing theanine (Unno, Iguchi et al., 2013). Normal diurnal rhythms of glucocorticoid are important for synapse formation in the brain (Liston et al., 2013). Stress-induced disruption of the diurnal rhythm of hormones is thought to contribute to reduced brain function. Theanine was shown to have stress-reducing effects by normalizing the HPA axis.

Adrenal hypertrophy due to confrontational rearing was observed in all strains of male mice examined to date. In the case of ddY mice, adrenals were maximal at 1 day after the start of confrontational rearing, while no significant enlargement was observed after 10 days (Unno, Iguchi et al., 2013). In contrast, adrenal hypertrophy was still observed at 7 months after the start of confrontational rearing in SAMP10 mice, a model of accelerated senescence (Unno et al., 2011). SAMP10 mice have a shorter lifespan than normal mice and show a decline in brain function and brain atrophy as they age (Takeda, 2009). Both ddY and SAMP10 mice showed a common stress response of adrenal hypertrophy at the beginning of confrontational rearing, indicating that both mice feel stress similarly, although the stress was reduced in ddY mice, and lasting longer in SAMP10 mice. Theanine showed greater inhibition of adrenal hypertrophy in both strains of mice. In female mice, the stress-loading method of confrontational rearing did not show any significant changes because they display low territoriality (Unno, Iguchi et al., 2013).

Stress-induced reduction in life span and stress suppression by theanine

The mean survival percentage of SAMP10 mice was significantly reduced under confrontational rearing conditions compared to normal rearing conditions in group rearing, and survival was reduced by 75% due to stress loading (Unno et al., 2011) (Table 61.1). However, even under the same stress conditions, survival was prolonged as much as in group-reared mice that had consumed water containing theanine (6 mg/kg). Feeding theanine to group-fed SAMP10 did not change mean survival, suggesting that theanine suppressed life span shortening by reducing stress.

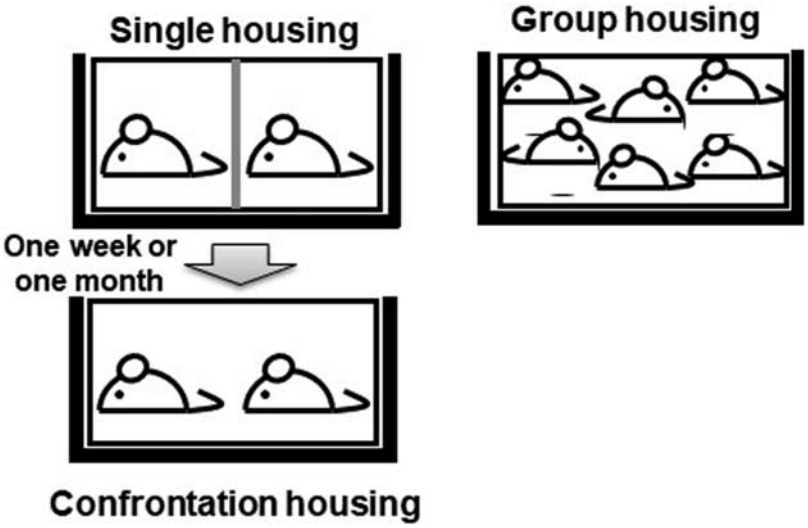

FIGURE 61.2 Rearing conditions. Mice were divided into confrontational and group-housing. In confrontational rearing, two randomly selected mice were separately housed in a partitioned cage that was divided into two identical subunits by a stainless steel partition. One month later, the partition was removed to expose the mice to confrontational stress and, subsequently, the two mice co-existed in a cage. Group-rearing mice were housed six per cage (Unno et al., 2011).

TABLE 61.1 Mean survival time (months) of SAMP10 mice.

Lifespan (months)	Group	Confrontation
Control	17.6 ± 1.5	13.6 ±1.5
Theanine	16.9 ± 1.4	17.9 ±1.4

The average survival rate of SAMP10 mice was measured under confrontational or group rearing. Mice in the theanine group received daily water containing theanine (20 μg/mL, 6 mg/kg). The control group consumed water without theanine. Values are mean ± SEM (n = 12) (Unno et al., 2011).

In contrast, no reduction in lifespan was observed in ddY mice. Taken together, these results suggest that SAMP10 is a stress-sensitive strain, while ddY is a stress-tolerant strain.

It is well known that there are individual differences in sensitivity to stress, with some individuals being more sensitive to stress than others, even under the same conditions. Although it is still not fully understood what causes these differences in stress sensitivity, these two strains of mice are considered to be suitable case animals for studying differences in stress sensitivity.

Stress-induced accelerated cognitive decline and oxidative brain damage

SAMP10 mice showed a significant decline in learning and memory ability after 11 months, but at 8 months of age, there was still no decline in learning and memory ability (Unno, Pervin et al., 2020). However, under confrontational rearing conditions, cognitive function already declined by 8 months of age, indicating that stress accelerated the decline in cognitive function (Unno et al., 2011). In contrast, no decline in brain function was observed in confrontation-reared mice fed theanine.

The brain consumes large amounts of oxygen and is susceptible to oxidative damage because it produces many reactive oxygen species (ROS) during metabolism (Taylor et al., 1999). To compare the extent of oxidative damage of cortical DNA, levels of 8-oxodeoxy-guanosine, as a marker of oxidative damage, were measured when mice were 9 months old. The results showed that oxidative damage increased significantly in mice of the same age in confrontational rearing compared to mice in group rearing (Unno et al., 2011).

Compared to normal mice, SAMP10 mice produce more ROS in the brain from a young age (Sasaki et al., 2008), and in aging mice, the activity of glutathione peroxidase, an antioxidant enzyme, is low (Kishido et al., 2007), indicating that oxidative damage to DNA tends to accumulate with age. However, unlike catechins, which exhibit strong antioxidant activity, theanine is thought not to exhibit direct antioxidant activity. The suppression of DNA oxidative damage by theanine intake is thought to be an indirect effect of maintaining the balance of ROS production/excretion in the brain.

Stress-induced brain atrophy and the effects of theanine

Until about a decade ago, no suitable experimental model for brain atrophy had been established, but experiments in SAMP10 mice revealed that not only the cerebrum atrophies with age, but that stress loading further accelerates brain atrophy (Unno et al., 2011). In order to clarify when stress-induced brain atrophy begins and in what areas it occurs, detailed brain measurements using nuclear magnetic resonance imaging (MRI) were carried out in mice that had been confrontationally reared for 1, 2, 4, and 6 months. The results indicate that SAMP10 mice showed significant atrophy of the cerebral cortex 1 month after the start of stress loading during confrontational rearing, followed by further atrophy (Fig. 61.3A) (Unno, Sumiyoshi et al., 2020). In the case of theanine-fed mice, significant atrophy was also observed after 1 month of stress load, but atrophy was once again restored at 2 months. In the hippocampus, a tendency to atrophy was observed after 1 month of stress loading, but the mice that consumed theanine subsequently recovered from atrophy and had a significantly larger hippocampus than mice in the control group that had not consumed theanine at 6 months.

In the ddY cortex, on the other hand, a trend toward atrophy was observed in the control group of mice 1 month after the start of confrontational rearing, but this subsequently recovered (Fig. 61.3B). No atrophy of the cerebral cortex was observed in theanine-fed mice. Similarly, no atrophy was observed in the hippocampus. These findings indicate that stress-induced brain atrophy occurs at an early stage and that theanine is involved in the suppression and recovery of stress-induced brain atrophy. In addition, SAMP10 and ddY mice showed differences in stress-induced brain atrophy.

Stress-induced changes in gene expression in the brain

The hippocampus is one of the most stress-sensitive tissues of the brain, and chronic stress has been shown to significantly reduce hippocampal volume and impair hippocampal neurogenesis in mice (Yun et al., 2010). Hippocampal neurogenesis occurs throughout life, regulates inhibitory

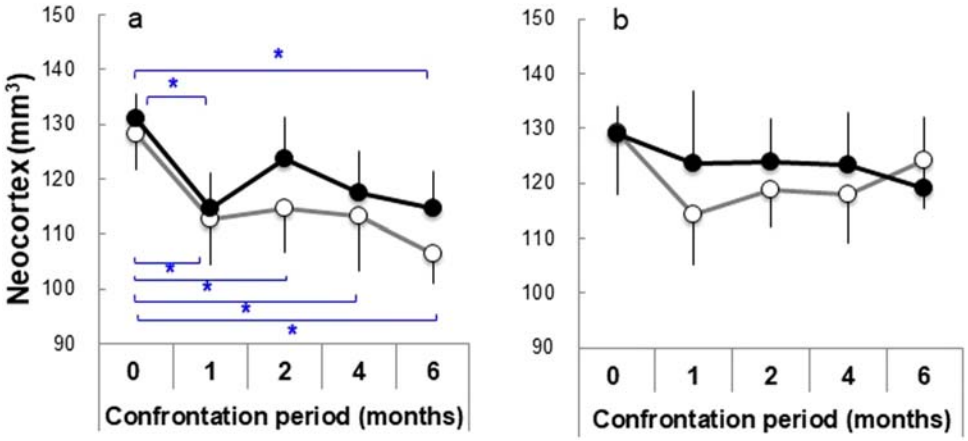

FIGURE 61.3 Brain volume of SAMP10 (A) and ddY (B) mice. Time-course of the neocortex was compared between control (*open circle*) and theanine (*closed circle*) (n = 3–12; *, $P < .05$) (Unno, Iguchi et al., 2020).

circuits, and may be associated with hippocampal-dependent learning and memory (Lieberwirth et al., 2016; Anacker et al., 2017). Since the effects of stress on the brain were observed early on, we comprehensively analyzed changes in gene expression in the hippocampus on the third day of stress loading by DNA microarray analysis in order to investigate what changes might be occurring in the brain.

Based on the results, changes over time were compared in detail by real-time PCR. The results showed that the expression of *neuronal PAS domain protein 4* (*Npas4*) was decreased significantly in the hippocampus of SAMP10 mice in the control group, but the decrease was suppressed in theanine-fed mice (Fig. 61.4) (Unno, Sumiyoshi et al., 2020). On the other hand, in ddY mice, *Npas4* expression increased somewhat in the control group mice, but it was slightly lowered by theanine intake. The transcription factor Npas4, an immediate early gene, is thought to play an important role in activity-dependent synapse formation and is closely related to anxiety, depressive-like behavior, and learning. Npas4 is known to be involved in the balance of neuronal excitation and inhibition. When rats were subjected to chronic stress, *Npas4* expression in the hippocampus was reduced, but its expression was restored in stress-tolerant rats compared to rats vulnerable to stress (Benatti et al., 2019). SAMP10 mice are vulnerable to stress, but theanine intake suppressed the decrease in *Npas4* expression, and this may have increased stress tolerance.

Lipocalin 2 (*Lcn2*) expression was markedly increased in SAMP10 mice after stress loading and was suppressed following the intake of theanine (Fig. 61.4) (Unno, Sumiyoshi et al., 2020). In contrast, its expression increased only slightly in ddY mice. Lcn2 is released from

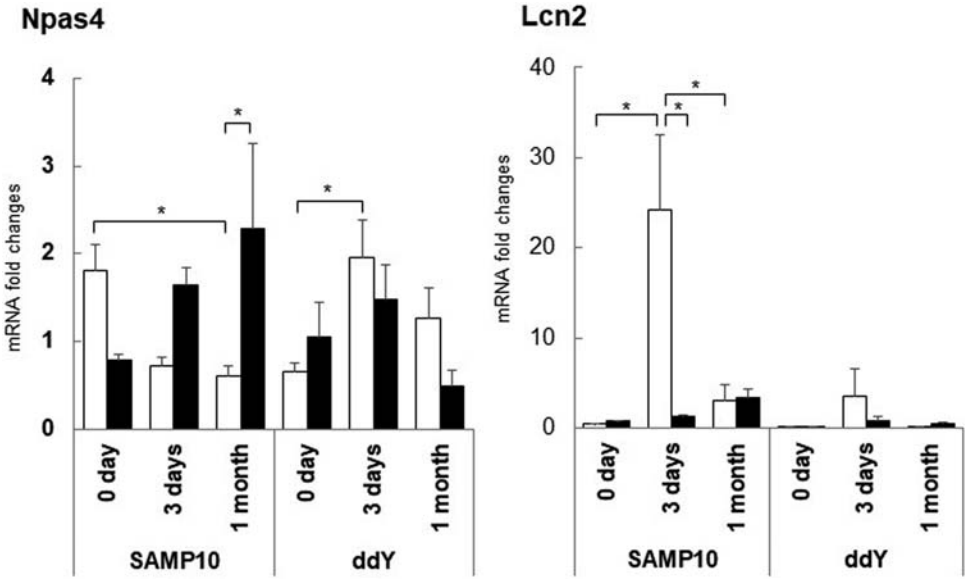

FIGURE 61.4 Expression levels of Npas4 and Lcn2 mRNA in the hippocampus of SAMP10 and ddY mice. Mice consumed theanine (20 μg/mL water, *closed bar*) or normal tap water (control, *open bar*) ad libitum. After single housing for 1 month, hippocampal samples were obtained from mice housed confrontationally for 0 days, 3 days, and 1 month. Values are expressed as means ± SEM (n = 3–6, *$P < .05$) (Unno, Iguchi et al., 2020).

astrocytes with an altered active form in various brain pathologies, causing nerve damage and increasing inflammation, which in turn increases neuronal cell death (Suk, 2016). Excessive *Lcn2* expression may be an important factor of brain atrophy in SAMP10 mice. Suppression of *Lcn2* expression by theanine may also be important in suppressing chronic inflammation associated with aging and neurodegenerative diseases.

Stress-induced changes in hippocampal metabolites and behavior

The effects of theanine intake were compared in a tail suspension test at 1 month after stress loading, when brain atrophy was significantly observed. The results showed that immobility time, which assesses depression-like behavior in SAMP10 and ddY mice, was significantly reduced in the theanine intake group, suggesting that theanine intake improves depression-like behavior in stress-loaded mice (Unno, Muguruma et al., 2020).

Among the statistically altered metabolites, kynurenine (Kyn) and histamine were significantly higher in SAMP10 than in ddY mice. Kyn is produced from tryptophan via indoleamine-2,3-dioxygenase (IDO), which was highly expressed in SAMP10 mice during stress loading. The high expression of IDO may contribute to the high Kyn levels in SAMP10 mice (Unno, Muguruma et al., 2020). The Kyn pathway plays an important role in depressive behavior in mice (Cattelan Souza et al., 2020; Zhang et al., 2020). Furthermore, Kyn increased in chronically stressed rats (Li et al., 2020).

Histamine, which is potentiated by various stressors, has a strong influence on hippocampal excitability (Brown et al., 2001). Theanine intake significantly suppressed histamine levels in stress-loaded SAMP10 mice (Unno, Muguruma et al., 2020), suggesting that the histaminergic system is an important target of theanine. Furthermore, theanine improved sleep quality (Rao et al., 2015). Histamine synthesis is regulated by inhibitory H3 autoreceptors on histamine neurons (Thakkar, 2011).

In contrast, carnosine, which is present in high concentrations in the brain, has antidepressant effects (Hipkiss, 2015; Tomonaga et al., 2008). Carnosine levels were significantly lower in SAMP10 mice than in ddY mice. Carnosine concentrations increased significantly in SAMP10 mice fed theanine even under confrontational rearing (Unno, Muguruma et al., 2020). The content of ornithine, which has antistress properties, was also significantly lower in SAMP10 mice than in ddY mice (Unno, Muguruma et al., 2020).

In the hippocampus of SAMP10 mice, stress load increased Kyn and histamine levels and decreased carnosine and ornithine levels. Theanine intake modulated these levels and improved depressive behavior, suggesting that these metabolic differences also contribute to stress vulnerability (Fig. 61.5).

Acceleration of brain aging due to psychosocial stress

In the stress-vulnerable SAMP10 mice, brain atrophy was observed at least 1 month after stress loading and further progressed with aging, resulting in reduced brain function, shortened lifespan, and accelerated aging. On the other hand, in stress-tolerant ddY mice, the degree of brain atrophy caused by stress load was not significant. Furthermore, this atrophy was restored and had no effect on brain function or lifespan.

Theanine treatment restored, although not completely, stress-induced brain atrophy in SAMP10 mice, while no atrophy was observed in ddY mice. In addition, theanine suppressed cognitive decline and shortened lifespan. Stress is thought to cause neuroinflammation, oxidative stress, excitation/inhibition imbalance, and inhibition of neurogenesis in the brain.

The relationship between stress and brain aging has been summarized in Fig. 61.6.

Stress reduction by theanine in a chronic overcrowding stress model

Whereas ddY mice were stress tolerant under confrontational rearing, stress sensitivity increased under long-term group rearing (Unno et al., 2023). ddY mice reared as six mice per cage gained approximately 1.5-fold body weight at 7 months of age compared to 1 month of age, suggesting that they accumulated overcrowding stress as they aged. However, stress was reduced in mice that ingested theanine.

Although suppression of excitability by increased expression of *Npas4* is important (Drouet et al., 2018), *Npas4* expression was suppressed in group-fed aged ddY mice. During transient stress, agonist-bound glucocorticoid receptor (holo-GR) represses *Npas4* expression in the brain by binding to the promoter (Furukawa-Hibi et al., 2012). In addition, prolonged stress loading causes decreased expression via DNA methylation at the promoter (Furukawa-Hibi et al., 2015). Indeed, holo-GR and a DNA methyltransferase (Dnmt3a) were elevated in group-fed aged mice but were suppressed in mice that ingested theanine (Fig. 61.7). Neural excitability also increases with age (Zullo et al., 2019), and thus the expression of *Npas4* increases, regulated by repressor element 1 silencing transcription factor (REST) (Bersten et al., 2014). Theanine was not associated with REST expression.

Stress reduction by theanine in the brain

Glutamate and γ-aminobutyric acid (GABA) are major excitatory and inhibitory neurotransmitters, respectively. During stress and stress-related disorders, the balance

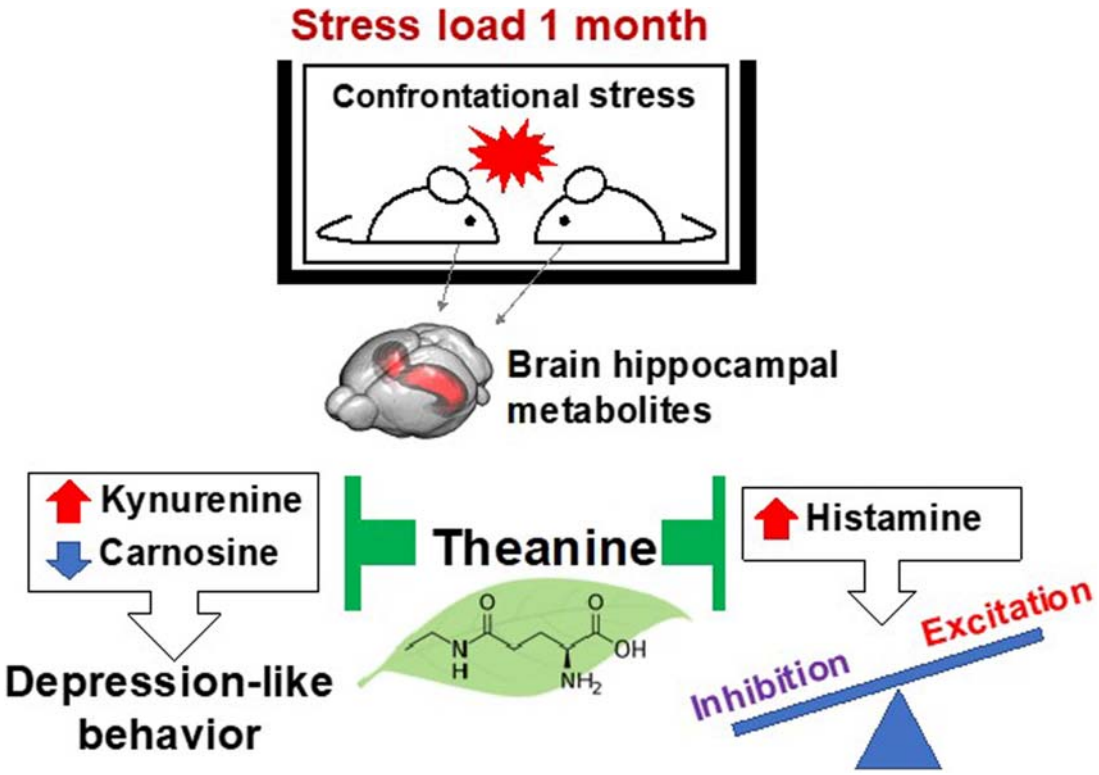

FIGURE 61.5 Changes in hippocampal metabolite levels in the hippocampus of SAMP10 mice. Mice were kept alone for 1 month and then under confrontational rearing for 1 month. Theanine (20 μg/mL, 6 mg/kg) or water (control) was fed for 2 months. As a result, kynurenine and histamine increased and carnosine decreased. Theanine suppressed these changes (Unno, Muguruma et al., 2020).

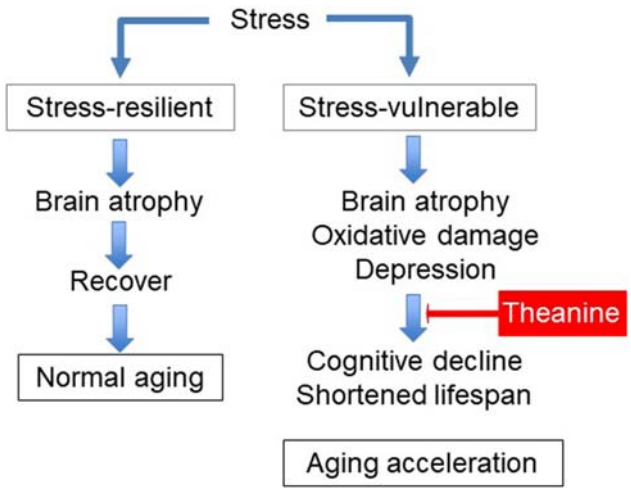

FIGURE 61.6 Relationship between stress and brain aging. In stress-sensitive mice (SAMP10), brain atrophy was observed at least 1 month after stress loading and then progressed further with aging. This resulted in accelerated aging (reduced brain function and shortened lifespan), which was inhibited by theanine. On the other hand, in stress-tolerant mice (ddY), the degree of stress-induced brain atrophy was not significant; furthermore, this atrophy was reversible, with no effect on brain function (Unno & Nakamura, 2021).

between GABA and glutamate in the brain becomes altered (Drouet et al., 2015). Theanine has been proposed to regulate the glutamate-glutamine cycle by binding to glutamine transporter and inhibiting overexcitation (Kakuda, 2011; Yoneda 2019). Theanine also acts on Npas4, which enhances GABA expression through the development of inhibitory synapses (Lin et al., 2008). Thus, theanine may modulate the balance between excitation and inhibition by acting on both GABA and glutamate levels (Fig. 61.8).

Stress-relieving effects of theanine in humans

Antistress effect of theanine

When the effect of drinking theanine on brain waves was examined, it was found that alpha waves, which indicate a relaxed state, appeared strongly after 30 min of drinking. Since few α waves were observed when the subjects simply drank water, theanine was found to have a relaxing effect (Kobayashi et al., 1998).

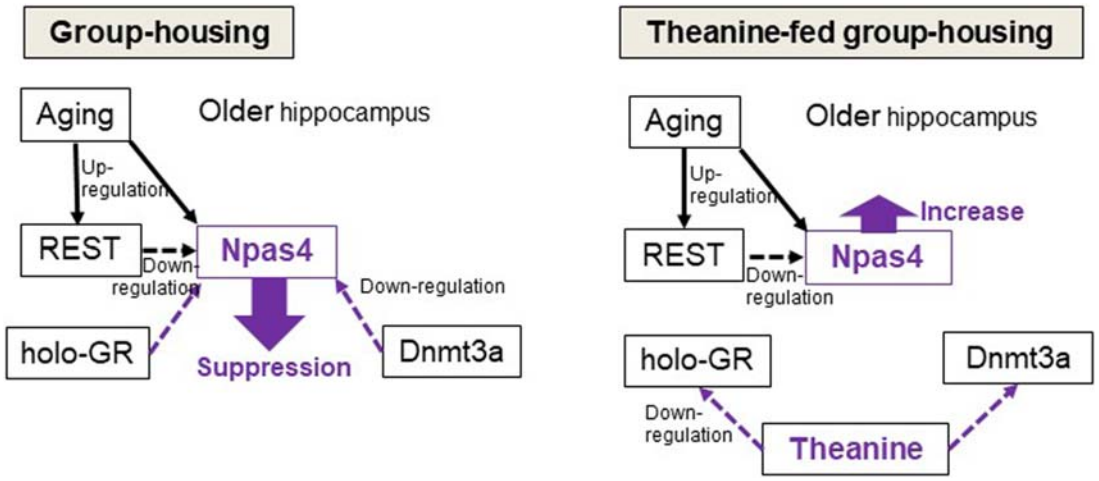

FIGURE 61.7 Factors relating to changes in Npas4 expression due to stress and aging, and the role of theanine. Holo-GR and Dnmt3a were upregulated in group-fed aged mice, suggesting that their elevated expression was involved in the downregulation of Npas4 expression. Theanine intake did not affect REST expression, but expression of holo-GR and Dnmt3a were significantly suppressed. *Solid line*: up-regulation. *Dashed line*: down-regulation (Unno et al., 2023).

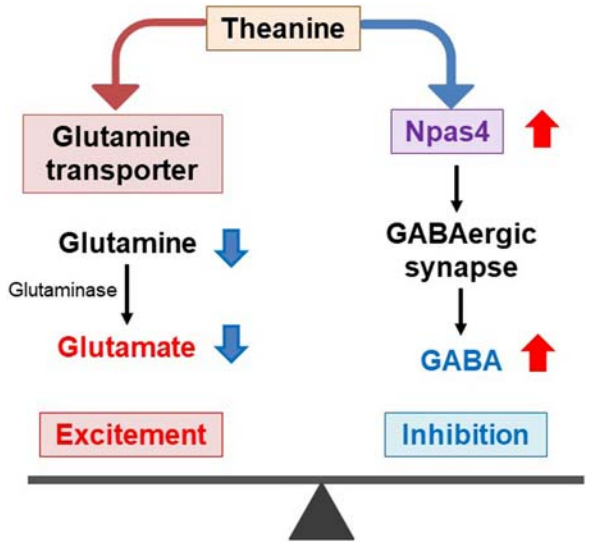

FIGURE 61.8 Factors relating to changes in Npas4 expression due to stress and aging, and the role of theanine. Theanine binds to glutamine transporter, regulates the glutamate-glutamine cycle, and inhibits over-excitation. Theanine also acts on Npas4 and enhances GABA expression via inhibitory synapses. In this way, theanine may regulate the balance between excitation and inhibition.

The effects of theanine (200 mg) intake on the transient mental stress load of a Kraepelin-type mental arithmetic task were evaluated in a crossover double-blind study using a placebo as the control (Kimura et al., 2007). Evaluations using Visual Analogue Scale (VAS) and State-Trait Anxiety Inventory (STAI) showed a significant reduction in subjective stress and anxiety. Significant increases in heart rate and salivary secretory IgA were also suppressed.

The effects of theanine (200 mg) ingestion on the transient mental stress task (the odoball task to auditory stimuli and an arithmetic test) were examined and found to be significantly reduced in the tension-anxiety item of the profile of mood states (Yoto et al., 2012). Theanine intake also significantly decreased systolic blood pressure in a group with higher poststress blood pressure.

Changes in salivary amylase activity are often used as an indicator of stress response. It was examined whether theanine intake (200 mg, twice daily) could relieve tension in students of pharmaceutical sciences during pharmacy practice (Unno, Tanida et al., 2013). The placebo group had higher salivary amylase activity in the morning, whereas the theanine group had the same activity as during daily college life. Individual differences in salivary amylase activity were also found, with students with higher salivary amylase activity having higher levels of trait anxiety as assessed by the STAI test.

Effects of theanine intake on sleep and depressed mood

In a study of healthy male subjects (25−36 years old), daily 200 mg of theanine (for 6 days) significantly improved the feeling of recovery from fatigue and subjective sleep duration as assessed by the Sleep Questionnaire in terms of the feeling of sleep upon waking. Sleep efficiency was also significantly improved as measured by an actigraph (Ozeki et al., 2004). On the other hand, it was also confirmed that theanine did not affect daytime sleepiness (Ozeki, Juneja et al., 2008).

The results of a study on the effects of daily 100 mg of theanine (for 1 week) on sleep in middle-aged healthy men (40−65 years old) using a simple sleep monitor (Sleep Scope, Sleep Well Co., Ltd., Osaka, Japan) showed no significant effects. However, an analysis that took into

account the involvement of background factors found that 100 mg of theanine increased non-REM sleep and improved sleep quality for those under 50 years of age or consuming less than three to four cups of green tea each week (Tominaga et al., 2022). However, coffee, which contains more caffeine than green tea, showed no effect even when consumed with three to four cups or less per week. This suggests that theanine's effect on sleep is influenced not only by age but also by the caffeine intake of coffee and green tea.

In a study of 8−12 year old boys, 200 mg of theanine twice daily (400 mg total) for 6 weeks significantly improved sleep percentage and sleep efficiency as measured by an actigraph (Lyon et al., 2011).

In a study of postmenopausal middle-aged elderly women (50−67 years old), pulse rate during sleep was analyzed using a wristwatch pulse wave sensor to examine the effects of 200 mg (6 days) of theanine intake. Theanine intake decreased sympathetic activity and increased parasympathetic activity during nocturnal sleep, suggesting that theanine may have improved sleep quality (Ozeki, Juneja et al., 2008).

When healthy men and women aged 20−69 years took theanine (200 mg/day for 4 weeks) and their sleep quality was assessed using the Pittsburgh Sleep Questionnaire, significant improvements in sleep quality, decreased depressed mood and anxiety, and improved cognitive function were reported (Hidese et al., 2019). Male and female patients (20−67 years old) with depression who took theanine (250 mg/day for 8 weeks) also reported improvements in depressive symptoms and anxiety as well as improved sleep (Hidese et al., 2017).

Patients with generalized anxiety disorder (18−75 years) who received theanine (450 mg/day for 4 weeks), then switched to 900 mg/day for another 4 weeks, did not show improvement in symptoms. No significant effects were observed in either the Hamilton Anxiety Rating Scale or the Insomnia Severity Index (ISI). However, patients who took theanine reported higher self-reported sleep satisfaction compared to placebo. In addition, the ISI of patients with nonclinical levels of insomnia symptoms showed an advantage for theanine intake (Sarris et al., 2019).

Mechanism of action of theanine on sleep

The importance of GABAergic neurons in sleep initiation and maintenance has been noted. Theanine intake increases the inhibitory transmitter GABA in the hippocampus while decreasing the major excitatory transmitter glutamate (Inoue et al., 2016). Theanine has also been reported to act via $GABA_A$ receptors (Egashira et al., 2007) and to increase GABA receptor expression (Dasdelen et al., 2022). Furthermore, the coexistence of GABA and theanine increases $GABA_A$ receptor expression (Kim et al., 2019). Taken together, it is predicted that theanine is involved in sleep initiation and maintenance via GABA and GABA receptors.

On the other hand, theanine has a high affinity for glutamine receptors (Kakuda, 2011) and is thought to affect the glutamate−glutamine cycle and consequently inhibit glutamate release. In addition, histamine is one of the components that promote arousal, while theanine intake decreases histamine levels in the hippocampus of stress-loaded mice (Unno, Muguruma et al., 2020). Furthermore, theanine intake increased serotonin in the brains of rats and mice (Dasdelen et al., 2022; Yokogoshi et al., 1998). Since serotonin is a precursor of the sleep hormone melatonin, it is possible that theanine may act on sleep through serotonin.

Stress-relieving effects of green tea

The amount of theanine in commercially available green teas such as Gyokuro and Matcha contain on average about 20 mg of theanine per gram of tea leaves, while a general green tea, Sencha, contains 10 mg, half of that amount. However, caffeine and epigallocatechin gallate (EGCG), a major catechin, counteract the stress-reducing effect of theanine, but in the case of Japanese green tea, arginine, the second most abundant amino acid after theanine, had the same stress-reducing effect as theanine (Unno et al., 2016). Thus, the stress reduction effect of Matcha can be estimated by the CE/TA ratio, which is the molar ratio of the sum of caffeine (C) and EGCG (E) to the sum of theanine (T) and arginine (A) (Unno et al., 2018). In animal experiments, the stress reduction effect was confirmed for Matcha with a CE/TA ratio of two or less. When students cooperated and consumed Matcha as cookies, Matcha with a CE/TA ratio of less than two was found to be more effective in relieving stress than placebo Matcha with a higher CE/TA ratio (Unno et al., 2019).

Summary points

1. There are individual differences in stress sensitivity.
2. In stress-sensitive mice, stress-induced aging is accelerated, but theanine suppresses it.
3. Theanine accelerates recovery from stress-induced brain atrophy.
4. Theanine suppresses stress-induced depression-like behavior.
5. Theanine is thought to reduce stress by regulating the balance between neuronal excitation and inhibition.
6. Theanine improves sleep.

References

Anacker, C., & Hen, R. (2017). Adult hippocampal neurogenesis and cognitive flexibility—Linking memory and mood. *Nature Reviews Neuroscience, 18*, 335−346.

Ansell, E. B., Rando, K., Tuit, K., Guarnaccia, J., & Sinha, R. (2012). Cumulative adversity and smaller gray matter volume in medial prefrontal, anterior cingulate, and insula regions. *Biological Psychiatry, 72*, 57−64.

Baba, Y., Inagaki, S., Nakagawa, S., Kaneko, T., Kobayashi, M., & Takihara, T. (2021). Effects of l-theanine on cognitive function in middle-aged and older subjects: A randomized placebo-controlled study. *Journal of Medicinal Food, 24*, 333−341.

Benatti, C., Radighieri, G., Alboni, S., Blom, J. M. C., Brunello, N., & Tascedda, F. (2019). Modulation of neuroplasticity-related targets following stress-induced acute escape deficit. *Behavioural Brain Research, 364*, 140−148.

Bersten, D. C., Wright, J. A., McCarthy, P. J., & Whitelaw, M. L. (2014). Regulation of the neuronal transcription factor NPAS4 by REST and microRNAs. *Biochimica et Biophysica Acta, 1839*, 13−24.

Brown, R. E., Stevens, D. R., & Haas, H. L. (2001). The physiology of brain histamine. *Progress in Neurobiology, 63*, 637−672.

Cattelan Souza, L., de Brito, M. L. O., Jesse, C. R., Boeira, S. P., de Gomes, M. G., Goes, A. T. R., Fabbro, L. D., Machado, F. R., Prigol, M., & Nogueira, C. W. (2020). Involvement of kynurenine pathway in depressive-like behaviour induced by nandrolone decanoate in mice. *Steroids, 164*, 108727.

Dar, T., Radfar, A., Abohashem, S., Pitman, R. K., Tawakol, A., & Osborne, M. T. (2019). Psychosocial stress and cardiovascular disease. *Current Treatment Options in Cardiovascular Medicine, 21*, 23.

Dasdelen, M. F., Er, S., Kaplan, B., Celik, S., Beker, M. C., Orhan, C., Tuzcu, M., Sahin, N., Mamedova, H., Sylla, S., Komorowski, J., Ojalvo, S. P., Sahin, K., & Kilic, E. (2022). A novel theanine complex, Mg-L-Theanine improves sleep quality via regulating brain electrochemical activity. *Frontiers in Nutrition, 9*, 874254.

Drouet, J. B., Fauvelle, F., Maunoir-Regimbal, S., Fidier, N., Maury, R., Peinnequin, A., Denis, J., Buguet, A., & Canini, F. (2015). Differences in prefrontal cortex GABA/glutamate ratio after acute restraint stress in rats are associated with specific behavioral and neurobiological patterns. *Neuroscience, 285*, 155−165.

Drouet, J. B., Peinnequin, A., Faure, P., Denis, J., Fidier, N., Maury, R., Buguet, A., Cespuglio, R., & Canini, F. (2018). Stress-induced hippocampus Npas4 mRNA expression relates to specific psychophysiological patterns of stress response. *Brain Research, 1679*, 75−83.

Egashira, N., Hayakawa, K., Osajima, M., Mishima, K., Iwasaki, K., Oishi, R., & Fujiwara, M. (2007). Involvement of GABA(A) receptors in the neuroprotective effect of theanine on focal cerebral ischemia in mice. *Journal of Pharmacological Sciences, 105*, 211−214.

Fujisawa, T. X., Shimada, K., Takiguchi, S., Mizushima, S., Kosaka, H., Teicher, M. H., & Tomoda, A. (2018). Type and timing of childhood maltreatment and reduced visual cortex volume in children and adolescents with reactive attachment disorder. *NeuroImage: Clinical, 20*, 216−221.

Furukawa-Hibi, Y., Nagai, T., Yun, J., & Yamada, K. (2015). Stress increases DNA methylation of the neuronal PAS domain 4 (Npas4) gene. *NeuroReport, 26*, 827−832.

Furukawa-Hibi, Y., Yun, J., Nagai, T., & Yamada, K. (2012). Transcriptional suppression of the neuronal PAS domain 4 (Npas4) gene by stress via the binding of agonist-bound glucocorticoid receptor to its promoter. *Journal of Neurochemistry, 123*, 866−875.

Hidese, S., Ogawa, S., Ota, M., Ishida, I., Yasukawa, Z., Ozeki, M., & Kunugi, H. (2019). Effects of L-theanine administration on stress-related symptoms and cognitive functions in healthy adults: A randomized controlled trial. *Nutrients, 11*, 2362.

Hidese, S., Ota, M., Wakabayashi, C., Noda, T., Ozawa, H., Okubo, T., & Kunugi, H. (2017). Effects of chronic l-theanine administration in patients with major depressive disorder: An open-label study. *Acta Neuropsychiatrica, 29*, 72−79.

Hipkiss, A. R. (2015). Possible benefit of dietary carnosine towards depressive disorders. *Aging and Disease, 6*, 300−303.

Inoue, K., Miyazaki, Y., Unno, K., Min, J. Z., Todoroki, K., & Toyo'oka, T. (2016). Stable isotope dilution HILIC-MS/MS method for accurate quantification of glutamic acid, glutamine, pyroglutamic acid, GABA and theanine in mouse brain tissues. *Biomedical Chromatography, 30*, 55−61.

Juruena, M. F., Eror, F., Cleare, A. J., & Young, A. H. (2020). The role of early life stress in HPA Axis and anxiety. *Advances in Experimental Medicine and Biology, 1191*, 141−153.

Kakuda, T. (2011). Neuroprotective effects of theanine and its preventive effects on cognitive dysfunction. *Pharmacological Research, 64*, 162−168.

Kakuda, T., Nozawa, A., Unno, T., Okamura, N., & Okai, O. (2000). Inhibiting effects of theanine on caffeine stimulation evaluated by EEG in the rat. *Bioscience Biotechnology & Biochemistry, 64*, 287−293.

Kim, S., Jo, K., Hong, K. B., Han, S. H., & Suh, H. J. (2019). GABA and l-theanine mixture decreases sleep latency and improves NREM sleep. *Pharmacien Biologiste, 57*, 65−73.

Kimura, K., Ozeki, M., Juneja, L. R., & Ohira, H. (2007). L-Theanine reduces psychological and physiological stress responses. *Biological Psychology, 74*, 39−45.

Kishido, T., Unno, K., Yoshida, H., Choba, D., Fukutomi, R., Asahina, S., Iguchi, K., Oku, N., & Hoshino, M. (2007). Decline in glutathione peroxidase activity is a reason for brain senescence: Consumption of green tea catechin prevents the decline in its activity and protein oxidative damage in ageing mouse brain. *Biogerontology, 8*, 423−430.

Kobayashi, K., Nagato, Y., Aoi, N., Juneja, L. R., Kim, M., Yamamoto, T., & Sugimoto, S. (1998). Effect of L-theanin on the release of α-brain waves in human volunteers. *Nippon Nogeikagaku Kaishi, 72*, 153−157.

Li, C. C., Jiang, N., Gan, L., Zhao, M. J., Chang, Q., Liu, X. M., & Pan, R. L. (2020). Peripheral and cerebral abnormalities of the tryptophan metabolism in the depression-like rats induced by chronic unpredicted mild stress. *Neurochemistry International, 138*, 104771.

Lieberwirth, C., Pan, Y., Liu, Y., Zhang, Z., & Wang, Z. (2016). Hippocampal adult neurogenesis: Its regulation and potential role in spatial learning and memory. *Brain Research, 1644*, 127−140.

Lin, Y., Bloodgood, B. L., Hauser, J. L., Lapan, A. D., Koon, A. C., Kim, T. K., Hu, L. S., Malik, A. N., & Greenberg, M. E. (2008). Activity-dependent regulation of inhibitory synapse development by Npas4. *Nature, 455*, 1198−1204.

Liston, C., Cichon, J. M., Jeanneteau, F., Jia, Z., Chao, M. V., & Gan, W. B. (2013). Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance. *Nature Neuroscience, 16*, 698−705.

Lyon, M. R., Kapoor, M. P., & Juneja, L. R. (2011). The effects of L-theanine (Suntheanine®) on objective sleep quality in boys with attention deficit hyperactivity disorder (ADHD): A randomized, double-blind, placebo-controlled clinical trial. *Alternative Medicine Review, 16*, 348−354.

Ota, M., Wakabayashi, C., Sato, N., Hori, H., Hattori, K., Teraishi, T., Ozawa, H., Okubo, T., & Kunugi, H. (2015). Effect of L-theanine on glutamatergic function in patients with schizophrenia. *Acta Neuropsychiatrica, 27*, 291−296.

Ozeki, M., Juneja, L. R., & Shirakawa, S. (2004). *The effects of L-theanine on sleep using the actigraph* (Vol. 9, pp. 143−150). Japan Society of Physiological Anthropology.

Ozeki, M., Juneja, L. R., & Shirakawa, S. (2008). *A study of L-theanine and daytime drowsiness* (Vol 13, pp. 9−15). Japan Society of Physiological Anthropology.

Ozeki, M., Juneja, L. R., & Shirakawa, S. (2008). *The effects of L-theanine on automatic nervous system during sleep in postmenopausal women* (Vol 13, pp. 147−154). Japan Society of Physiological Anthropology.

Rao, T. P., Ozeki, M., & Juneja, L. R. (2015). In search of a safe natural sleep aid. *Journal of the American College of Nutrition, 34*, 436−447.

Sarris, J., Byrne, G. J., Cribb, L., Oliver, G., Murphy, J., Macdonald, P., Nazareth, S., Karamacoska, D., Galea, S., Short, A., Ee, C., Birling, Y., Menon, R., & Ng, C. H. (2019). L-theanine in the adjunctive treatment of generalized anxiety disorder: A double-blind, randomised, placebo-controlled trial. *Journal of Psychiatric Research, 110*, 31−37.

Sasaki, T., Unno, K., Tahara, S., Shimada, A., Chiba, Y., Hoshino, M., & Kaneko, T. (2008). Age-related increase of superoxide generation in the brains of mammals and birds. *Aging Cell, 7*, 459−469.

Suk, K. (2016). Lipocalin-2 as a therapeutic target for brain injury: An astrocentric perspective. *Progress in Neurobiology, 144*, 158−172.

Takeda, T. (2009). Senescence-accelerated mouse (SAM) with special references to neurodegeneration models, SAMP8 and SAMP10 mice. *Neurochemical Research, 34*, 639−659.

Taylor, D. L., Edwards, A. D., & Mehmet, H. (1999). Oxidative metabolism, apoptosis and perinatal brain injury. *Brain Pathology, 9*, 93−117.

Terashima, T., Takido, J., & Yokogoshi, H. (1999). Time-dependent changes of amino acids in the serum, liver, brain and urine of rats administered with theanine. *Bioscience Biotechnology & Biochemistry, 63*, 615−618.

Thakkar, M. M. (2011). Histamine in the regulation of wakefulness. *Sleep Medicine Reviews, 15*, 65−74.

Tominaga, T., Furushima, D., Unno, K., Sugiyama, I., Ozeki, M., Nakamura, Y., & Yamada, H. (2022). An exploratory evaluation of a low dose theanine consumption on improving sleep in middle-aged and older males. *Food Science & Nutrition, 5*, 1.

Tomonaga, S., Yamane, H., Onitsuka, E., Yamada, S., Sato, M., Takahata, Y., Morimatsu, F., & Furuse, M. (2008). Carnosine-induced antidepressant-like activity in rats. *Pharmacology Biochemistry and Behavior, 89*, 627−632.

Unno, K., Fujitani, K., Takamori, N., Takabayashi, F., Maeda, K., Miyazaki, H., Tanida, N., Iguchi, K., Shimoi, K., & Hoshino, M. (2011). Theanine intake improves the shortened lifespan, cognitive dysfunction and behavioural depression that are induced by chronic psychosocial stress in mice. *Free Radical Research, 45*, 966−974.

Unno, K., Furushima, D., Hamamoto, S., Iguchi, K., Yamada, H., Morita, A., Horie, H., & Nakamura, Y. (2018). Stress-reducing function of matcha green tea in animal experiments and clinical trials. *Nutrients, 10*, 1468.

Unno, K., Furushima, D., Hamamoto, S., Iguchi, K., Yamada, H., Morita, A., Pervin, M., & Nakamura, Y. (2019). Stress-reducing effect of cookies containing matcha green tea: Essential ratio among theanine, arginine, caffeine and epigallocatechin gallate. *Heliyon, 5*, e01653.

Unno, K., Hara, A., Nakagawa, A., Iguchi, K., Ohshio, M., Morita, A., & Nakamura, Y. (2016). Anti-stress effects of drinking green tea with lowered caffeine and enriched theanine, epigallocatechin and arginine on psychosocial stress induced adrenal hypertrophy in mice. *Phytomedicine, 23*, 1365−1374.

Unno, K., Iguchi, K., Tanida, N., Fujitani, K., Takamori, N., Yamamoto, H., Ishii, N., Nagano, H., Nagashima, T., Hara, A., Shimoi, K., & Hoshino, M. (2013). Ingestion of theanine, an amino acid in tea, suppresses psychosocial stress in mice. *Experimental Physiology, 98*, 290−303.

Unno, K., Muguruma, Y., Inoue, K., Konishi, T., Taguchi, K., Hasegawa-Ishii, S., Shimada, A., & Nakamura, Y. (2020). Theanine, antistress amino acid in tea leaves, causes hippocampal metabolic changes and antidepressant effects in stress-loaded mice. *International Journal of Molecular Sciences, 22*, 193.

Unno, K., & Nakamura, Y. (2021). Green tea suppresses brain aging. *Molecules, 26*, 4897.

Unno, K., Pervin, M., Taguchi, K., Konishi, T., & Nakamura, Y. (2020). Green tea catechins trigger immediate-early genes in the hippocampus and prevent cognitive decline and lifespan shortening. *Molecules, 25*, 1484.

Unno, K., Sumiyoshi, A., Konishi, T., Hayashi, M., Taguchi, K., Muguruma, Y., Inoue, K., Iguchi, K., Nonaka, H., Kawashima, R., Hasegawa-Ishii, S., Shimada, A., & Nakamura, Y. (2020). Theanine, the main amino acid in tea, prevents stress-induced brain atrophy by modifying early stress responses. *Nutrients, 12*, 174.

Unno, K., Taguchi, K., Konishi, T., Ozeki, M., & Nakamura, Y. (2023). Theanine, a tea-leaf-specific amino acid, alleviates stress through modulation of Npas4 expression in group-housed older mice. *International Journal of Molecular Sciences, 24*, 3983.

Unno, K., Tanida, N., Ishii, N., Yamamoto, H., Iguchi, K., Hoshino, M., Takeda, A., Ozawa, H., Ohkubo, T., Juneja, L. R., & Yamada, H. (2013). Anti-stress effect of theanine on students during pharmacy practice: Positive correlation among salivary α-amylase activity, trait anxiety and subjective stress. *Pharmacology Biochemistry and Behavior, 111*, 128−135.

Yamada, T., Terashima, T., Kawano, S., Furuno, R., Okubo, T., Juneja, L. R., & Yokogoshi, H. (2009). Theanine, gamma-glutamylethylamide, a unique amino acid in tea leaves, modulates neurotransmitter concentrations in the brain striatum interstitium in conscious rats. *Amino Acids, 36*, 21−27.

Yokogoshi, H., Kobayashi, M., Mochizuki, M., & Terashima, T. (1998). Effect of theanine, r-glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats. *Neurochemical Research, 23*, 667−673.

Yoneda, Y., Kawada, K., & Kuramoto, N. (2020). Selective upregulation by theanine of Slc38a1 expression in neural stem cell for brain wellness. *Molecules, 25*, 347.

Yoneda, Y., Kuramoto, N., & Kawada, K. (2019). The role of glutamine in neurogenesis promoted by the green tea amino acid theanine in neural progenitor cells for brain health. *Neurochemistry International, 129*, 104505.

Yoto, A., Motoki, M., Murao, S., & Yokogoshi, H. (2012). Effects of L-theanine or caffeine intake on changes in blood pressure under physical and psychological stresses. *Journal of Physiological Anthropology, 31*, 28.

Yun, J., Koike, H., Ibi, D., Toth, E., Mizoguchi, H., Nitta, A., Yoneyama, M., Ogita, K., Yoneda, Y., Nabeshima, T., et al. (2010). Chronic restraint stress impairs neurogenesis and hippocampus-dependent fear memory in mice: Possible involvement of a brain-specific transcription factor Npas4. *Journal of Neurochemistry, 114*, 1840−1851.

Zhang, Q., Sun, Y., He, Z., Xu, Y., Li, X., Ding, J., Lu, M., & Hu, G. (2020). Kynurenine regulates NLRP2 inflammasome in astrocytes and its implications in depression. *Brain, Behavior, and Immunity, 88*, 471−481.

Zullo, J. M., Drake, D., Aron, L., O'Hern, P., Dhamne, S. C., Davidsohn, N., Mao, C. A., Klein, W. H., Rotenberg, A., Bennett, D. A., et al. (2019). Regulation of lifespan by neural excitation and REST. *Nature, 574*, 359−364.