
Role of Green Tea in Brain Aging

Keiko Unno ^{a*} and Yoriyuki Nakamura ^a

DOI: 10.9734/bpi/ctcb/v2/2647B

ABSTRACT

Epidemiological studies have demonstrated that the intake of green tea is effective for reducing the risk of dementia. However, it is not clear which components of green tea reduce that risk, and how they act. The most important risk factor for dementia is brain aging. Therefore, our research has focused on the role of green tea in preventing brain aging. Epigallocatechin gallate (EGCG) is the most significant component in green tea. Although both EGCG and epigallocatechin (EGC) have been shown to pass the blood–brain barrier and reach the brain parenchyma, EGCG has been proven to be more effective at promoting neuronal differentiation than EGC. It's also been proposed that the products of EGCG deconstruction by the gut microbiota enhance nerve cell development, and that both EGCG and its degradation products act on nerve cells, albeit with a time lag. On the other hand, the free amino acids theanine and arginine present in green tea have stress-reducing effects. While long-term stress accelerates aging of the brain, theanine and arginine suppress this aging due to their anti-stress effect. Since this effect is counteracted by EGCG and caffeine, the ratio of these green tea components is important for the anti-stress action. Based on these findings, it is thought that the suppression of brain aging by green tea through activation of neurons by EGCG and its degradation products, and the reduction of stress by theanine and arginine, may be involved in reducing the risk of dementia.

Keywords: Arginine; brain aging; catechin; epigallocatechin gallate; green tea; stress reduction; theanine.

1. INTRODUCTION

Since the aging population is growing worldwide, brain aging is a universal problem. The number of elderly people in Japan is quickly increasing, and the country has the largest percentage of elderly persons in the world. According to data obtained in 2012 by a research group from Japan's Ministry of Health, Labor, and Welfare, over 70% of women aged 95 and up had dementia. Similarly, more than half of men aged 95 and up suffer from dementia. Apart from genetic considerations, brain aging, as well as environmental and lifestyle-related factors, all have a role in dementia. Improving these aspects and mitigating age-related changes are believed to help to delay the onset of dementia. With age, various changes, such as arteriosclerosis, climacteric disorder, diabetes, hyperlipidemia, kidney disease, hypertension, obesity, and poor memory, commonly occur. However, these age-related changes have reportedly been improved or counteracted in people who followed advice pertaining to exercise and diet [1]. Ingesting green tea is one such recommendation. In fact, the consumption of green tea has been epidemiologically shown to be inversely associated with both male and female heart disease mortality and male cerebrovascular and respiratory mortality [2]. In addition, there are accumulating reports indicating that the ingestion of green tea is effective in preventing dementia [3–7]. In order to elucidate the effect of green tea, studies mainly focusing on the antioxidant effect of epigallocatechin gallate (EGCG) have been conducted in vivo and in vitro [8–11]. The effects of EGCG are often strong, but the effects of green tea cannot always be explained exclusively by the antioxidant effects of EGCG. It is also necessary to investigate the interactions among other green tea components, such as caffeine, theanine, and arginine. In addition, the involvement of green tea metabolites must also be studied. Detailed studies have been conducted on the metabolites of green

^a Tea Science Center, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan.

*Corresponding author: E-mail: unno@u-shizuoka-ken.ac.jp;

tea catechins [12–14], and research on their biological effects has been conducted in recent years [15–21]. It should be noted that the metabolites produced by the gut microbiota have important effects on human health. The most potent green tea catechin is epigallocatechin-3-gallate, which is responsible for a wide range of health benefits including anticancer, antidiabetics, and anti-inflammatory properties. However, green tea catechins (GTCs) are very labile under both environmental and gastrointestinal conditions; their chemical stability and bioavailability primarily depend on the processing and formulation conditions [114–116].

Theanine, the most abundant free amino acid in green tea, has been found to relieve stress and has a relaxing effect [22–26]. In addition, arginine, which is the second most abundant free amino acid in Japanese green tea, has an anti-stress effect, similar to theanine [27,28]. However, since the stress-reducing effects of theanine and arginine are counteracted by EGCG and caffeine, the compositional ratio of these components determines the anti-stress effect of green tea [27,29]. Stress is deeply involved in the development and progression of many diseases, such as asthma, hypertension, peptic ulcers, obesity, and diabetes. The brain is also subjected to significant stress. Even healthy people undergo atrophy in the brain when they are repeatedly subjected to intense stress—for example, as a result of bereavement, job loss, accidents, or major natural disasters [30–32]. It has also been reported that brain atrophy occurs in abused children [33–35]. Furthermore, many people are more stressed than before due to the current coronavirus pandemic [36,37]. The stress-reducing effect of theanine and green tea is thought to contribute to the maintenance of mental health and the control of brain aging.

In order to clarify the functionality of foods, the interactions among food components and the actions of metabolic decomposition products must be examined. In this chapter, we focus on the effects of green tea components—catechins, EGCG metabolites, theanine, and arginine—on brain function. In addition, we explain the anti-stress and anti-depressant effects of several types of green tea.

2. CATECHINS

Among the tea catechins, epicatechin was first isolated from green tea (*Camellia sinensis* (L.) O. Kuntze) extract by Michiyo Tsujimura in 1930 [38]. Catechin can assume four different structures: (+) catechin, (–) catechin, (+) epicatechin, and (–) epicatechin; the (–) epi-form is most common in green tea. In addition, gallic acid is found in catechins and gallo catechins, and (–) epigallocatechin gallate (EGCG) is the most abundant catechin in green tea. (–) Epigallocatechin (EGC), (–) epicatechin gallate (ECG), and (–) epicatechin (EC) are also found (Fig. 1). Unlike normal flavonoids, catechins rarely exist in nature as a sugar-bound glycoside. Although catechins are also found in arboreal fruits such as cacao, grapes, apples, and peaches, their main components are free catechins, such as (+) catechin, (–) epicatechin, and (–) epigallocatechin, that do not contain gallic acid. Therefore, gallate-type catechins such as EGCG and ECG are unique to tea leaves.

2.1 Lifespan

Brain aging does not always coincide with body aging, but lifespan is an important marker of body aging. It has been confirmed that green tea or EGCG has a life-prolonging effect on nematodes, flies, and mice [39–41]. According to the results of an epidemiological survey of approximately 91,000 Japanese people aged between 40 and 69, who were followed for 18.7 years, all-cause mortality was significantly reduced in those who drank five or more cups of green tea every day, compared to those who drank less than one cup per day [2]. Similarly, elderly Chinese men who consumed tea almost every day showed a 10–20% lower risk of death compared to their counterparts who seldom consumed tea [42].

The relationship between the intake of green tea catechins and lifespan was investigated using senescence-accelerated model mice, SAMP10/TaSlc (SAMP10), which have a short lifespan. The median survival time (MST) was significantly prolonged when 1 mg/kg (equivalent to one cup of green tea per day in humans) was consumed daily [41]. Even when 5 to 60 mg/kg of green tea catechins were consumed, MST was prolonged, although not significantly, when compared with mice that

consumed no green tea catechins [41]. It thus seems important to drink several cups of green tea at least daily, but not to consume large amounts of catechins.

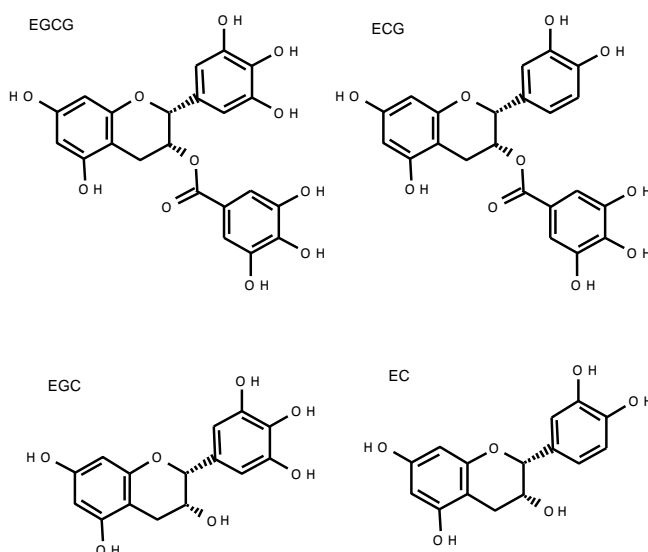


Fig. 1. The structure of green tea catechins

2.2 Cognitive Function

The intake of green tea has been reported to prevent cognitive decline [3,4,43,44]. In experiments using SAMP10, we found that the daily ingestion of green tea catechins suppressed brain dysfunction in aged mice [45,46]. To clarify the effect of green tea catechins on the suppression of cognitive impairment with aging, the effect of the start time of intake of green tea catechins was investigated in aged SAMP10 mice. Six- or nine-month-old SAMP10 mice initially drank water containing green tea catechins until they were 11 months old [47]. When SAMP10 mice are six and nine months of age, they are considered to be adults and middle-aged, respectively. Mice that received green tea catechins between the age of 6 and 11 months had significantly higher memory acquisition capacity, as examined by passive avoidance trials, than control mice of the same age who did not consume green tea catechins [47]. Consuming green tea catechins between the age of 9 and 11 months also tended to improve learning ability [47]. The daily ingestion of green tea catechins has been suggested to suppress brain dysfunction with aging. Additionally, even if ingestion started from middle age, this appeared to be more beneficial than not consuming it at all.

Next, the dose-dependency of the effect of green tea catechins on cognitive function was examined. A significant effect on memory acquisition was observed at 1 mg/kg or more of daily ingestion of green tea catechins, as assessed by the passive avoidance test, and the most effective dose was 15 mg/kg [41]. Long-term memory retention was significantly higher in mice that consumed daily 60 mg/kg of green tea catechins than control. Spatial working memory measured using a Y-maze improved significantly in mice that ingested at least 30 mg/kg of green tea catechins [41]. In other words, the suppression of cognitive decline by green tea catechins was dependent on dose, with a minimum required daily dose of 1 mg/kg in mice (equivalent to one cup of green tea in humans).

The ability of two catechins EGCG or EGC to suppress the decline in cognitive function was compared, but no effect was observed by EGC at the same concentration (0.1mM) [48]. The difference between EGCG and EGC is epitomized by the presence or absence of a galloyl moiety, although gallic acid (GA) itself had no effect [48]. Regarding the action of catechins, the importance of antioxidative activity has been highlighted. In fact, in mice that ingested the green tea catechins EGCG or ECG, lipid peroxidation in the cerebral cortex was significantly reduced compared to the control group, indicating that antioxidant capacity was not significantly different between EGCG and

EGC [48]. In other words, it is difficult to completely explain the difference between EGCG and EGC on the suppression of cognitive decline based on antioxidative activity alone.

2.3 Absorption and Metabolism of Catechins

When EGCG is orally ingested, a small amount is taken up from the small intestine and enters the systemic circulation without any degradation [49,50], and only a trace amount remains eight hours after ingestion. On the other hand, most EGCG is decomposed into EGC and GA by the intestinal microbiota [51]. Degalloylated catechins are further degraded in the large intestine, and converted into 5-(3',5'-dihydroxyphenyl)- γ -valerolactone (EGC-M5) (Fig. 2), 5-(3',4'-dihydroxyphenyl)- γ -valerolactone, and 5-(3',4',5'-trihydroxyphenyl)- γ -valerolactone as the major degradation products more than eight hours after ingestion [13,14,52]. These valerolactones are readily absorbed into the large intestine and subsequently metabolized and distributed [13]. The conjugated forms of these catechins and metabolites, such as sulfates (EGC-M5-Sul) and glucuronides (EGC-M5-GlcUA), have been found in plasma and urine [13]. GA becomes a glucuronide conjugate (PG-GlcUA) via pyrogallol (PG) [51] (Fig. 2).

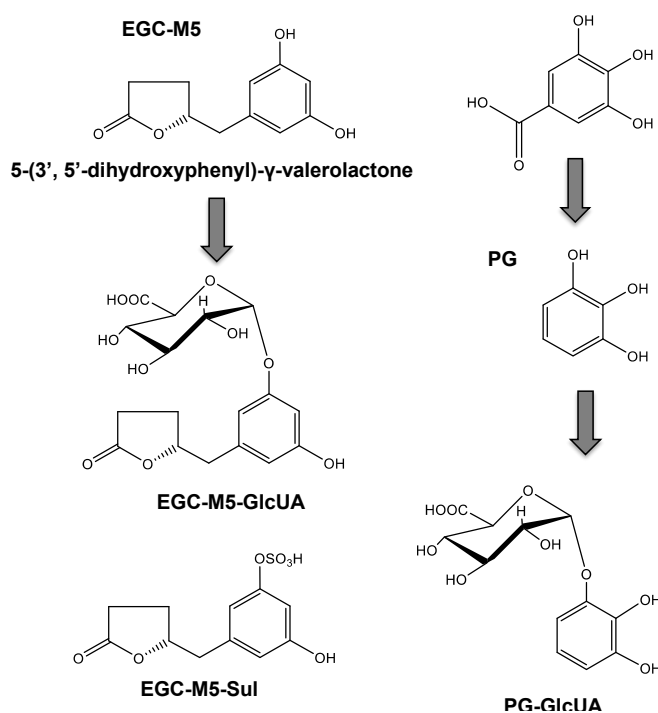


Fig. 2. The structure of EGCG metabolites

2.4 Blood–Brain Barrier Permeability of Catechins and Their Degradation Products

Since catechins and their degradation products need to cross the blood–brain barrier (BBB) in order to act directly in the brain, permeability from the vascular side to the parenchyma side of the brain was measured using an in vitro BBB assay kit (RBT-24, Pharmaco-Cell Co. Ltd., Nagasaki, Japan). The permeability of EGCG was 4% and that of EGC was 5% in 30 min [17,48] (Table 1). These values are lower than the permeabilities of caffeine and GA, but catechins such as EGCG and EGC undoubtedly reached the brain parenchyma. Using a similar in vitro BBB model, the transfer rates of (+) catechin and (–) epicatechin were reported as 7.4% and 15.4% in 1 h, respectively [53]. This indicates that the difference in the configuration of the hydroxyl group at the 3-position of the flavan structure greatly affects BBB permeability. EGCG has a large galloyl group at the 3-position, but the high permeability of GA is believed to enhance the permeability of EGCG to a level similar to EGC. EGC-M5 showed slightly higher permeability than EGCG and EGC. EGC-M5 conjugates were less permeable than EGC-M5, while EGC-M5-Sul showed slightly higher values than EGCG [17]. These data indicate that

the effects of both catechins and their degradation products in the brain should be investigated. On the other hand, when EGC and GA were present together, the permeability of EGC was considerably reduced compared to EGC alone. Further research is needed to evaluate whether such competitive effects actually occur in the brain.

Table 1. BBB permeability of green tea catechins and their metabolites

Sample	Coexistence sample	Permeability coefficient (10^{-6} cm/s)	BBB permeability (%) (0.5 h)
EGCG		13.45 ± 0.57	4.00 ± 0.17
EGCG	EGC	10.53 ± 0.63	3.13 ± 0.19
EGC		16.70 ± 1.86	4.96 ± 0.55
EGC	EGCG	12.39 ± 1.56	3.68 ± 0.47
EGC	GA	6.01 ± 1.57	1.79 ± 0.47
GA		31.73 ± 3.39	9.42 ± 1.01
GA	EGC	26.98 ± 2.77	8.01 ± 0.82
EGC-M5		17.99 ± 0.79	5.34 ± 0.23
EGC-M5-Sul		14.61 ± 1.35	4.34 ± 0.40
EGC-M5-GlcUA		12.53 ± 0.02	3.72 ± 0.01
Pyrogallol		13.79 ± 1.62	4.10 ± 0.48
Pyro-GlcUA		9.28 ± 1.41	2.76 ± 0.42
Caffeine		45.22 ± 4.41	13.43 ± 1.31

Data are expressed as the mean ± SEM (n = 3). (Data of Ref. 17 and 48 have been modified).

2.5 Effect of Green Tea Catechins on SH-SY5Y Cells

SH-SY5Y, a human neuroblastoma, was used to investigate the action of green tea catechins that enter the brain parenchyma. When SH-SY5Y cells were treated with 50 nM of catechins, the neurites, which are markers of nerve cell differentiation, became longer, and their number increased. The action of EGCG was stronger than that of EGC and GA (Fig. 3) [48]. The effect of EGC-M5 on SH-SY5Y cells was the second strongest after EGCG [17]. EGC-M5-Sul also had a neurite-stretching effect [17]. Interestingly, it was reported that when 5-(3',4'-dihydroxyphenyl)- γ -valerolactone was administered to rats, the sulfate-conjugated 5-(3',4'-dihydroxyphenyl)- γ -valerolactone was detected in the brain, but not in the form of aglycones [15]. Therefore, EGC-M5-Sul may play an important role in the brain as an EGCG degradation product.

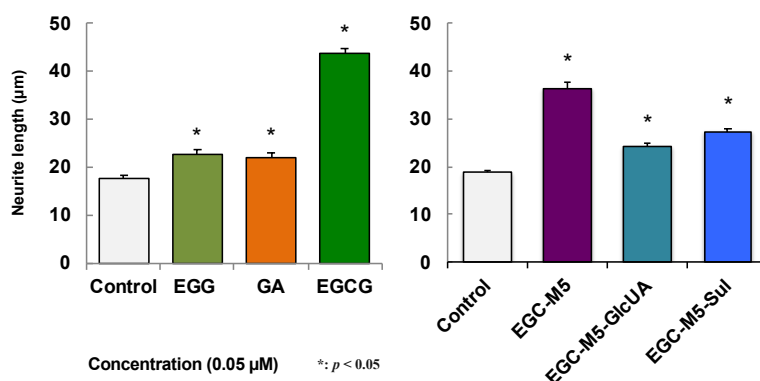


Fig. 3. The neurite outgrowth of human SH-SY5Y cells treated with EGCG and its metabolites. A cell suspension (2.5×10^4 cells/well) was plated in a 24-well plate. Samples dissolved in 0.01% DMSO were added to the culture medium to produce a final concentration of 0.05 μ M, and these were cultured for 72 h at 37°C. Each value represents the mean ± SEM (neurite length, n = 103-107). Asterisks represent significant differences relative to the control (* p < 0.05, Bonferroni's t-test) [17,48]

2.6 Altered Gene Expression Due to Ingestion of Green Tea Catechins

In order to elucidate the mechanism by which the intake of green tea catechins suppresses the decline in brain function, we investigated which genes' expression changed in the brain. The hippocampus of SAMP10 mice treated with green tea catechins for one month displayed significantly higher expression of some of the immediate early genes (IEGs), such as nuclear receptor subfamily 4, group A, member 1 (*Nr4a1*), FBJ osteosarcoma oncogene (*Fos*), early growth response 1 (*Egr1*), neuronal PAS domain protein 4 (*Npas4*), and cysteine-rich protein 61 (*Cyr61*) [41]. The expression of IEGs is first induced in response to extracellular stimuli and is widely used as a marker of neural activity. *Fos*, *Egr-1*, and *Npas4* play key roles in long-term synaptic plasticity [54]. In addition, *Npas4* regulates the excitatory and inhibitory balance within circuits. *Nr4a1* plays a role in regulating the density and distribution of spines and synapses [55], and in suppressing the age-related decline in brain function [56]. *Cyr61* is needed for dendritic arborization in the hippocampus [57]. The higher expression of these IEGs in the hippocampus is thought to increase synaptic plasticity, contributing to the maintenance and improvement of learning and memory abilities. The transcription of many IEGs in neurons is initiated by the influx of calcium ion associated with synaptic activity and action potential [58]. Moreover, EGCG modulates calcium signals in hippocampal neurons [59,60]. Therefore, EGCG may increase the expression of IEGs by increasing the levels of intracellular calcium ions in hippocampal nerve cells.

2.7 Actions of EGCG and its Degradation Products on the Brain

Since the transfer of catechins to the brain parenchyma is restricted by the BBB, the amount of catechins in the brain is considered to be lower than in the periphery. The concentration-dependent effect of green tea catechins on cognitive function was observed in mice, but it was suggested that a daily intake of 1 mg/kg or more of green tea catechins suppresses age-related cognitive decline [41]. Epidemiological studies have also reported that a daily intake of one or several cups of green tea reduces the risk of dementia [3,4,42–44]. The elution of catechins into water is temperature dependent and they are stable in hot water. The quantity of catechins contained in green tea is generally around 70 mg/100 mL when eluted with hot water from tea leaves, and 30–50 mg/100 mL in commercially available bottles of green tea. Based on the BBB permeability of catechins, as discussed in Section 2.4, the concentration of EGCG and its degradation products will presumably promote the differentiation of nerve cells when several cups of green tea, or approximately one bottle of catechins, are consumed per day. This may reduce the age-related decline in cognitive function.

We hypothesize that by drinking a few cups of green tea every day, both EGCG and its degradation products may be taken up in the brain and activate nerve cells, as summarized below and in Fig. 4.

1. Two to three hours after ingesting green tea, a very small amount of EGCG is taken up from the small intestine and enters the brain parenchyma through the blood-stream.
2. The incorporated EGCG activates nerve cells by enhancing IEG expression and promoting cell differentiation. After this, EGCG is excreted.
3. On the other hand, in the large intestine, most EGCG is decomposed from EGC into valerolactones, such as EGC-M5, by the intestinal microbiota over a period of 8 h.
4. The resulting EGC-M5 (and other decomposed products) is taken up from the large intestine.
5. EGC-M5 and its conjugates are delivered to the brain via systemic circulation.
6. Nerve cells are reactivated.

In this way, by drinking a few cups of green tea every day, brain aging might be slowed. The bioavailability of catechins was thought to be low, but in fission metabolites, it has been reported to be approximately 40 to 62%, albeit with large variability among individuals [52,61]. It has been pointed out that phenylvalerolactone and phenylvaleroic acid are important as metabolic fission products common to flavan-3-ols [52]. In addition to the effects of EGCG degradation products on nerve cells, beneficial effects on blood pressure, immunity, and glucose metabolism have been reported [18–20]. The physiological effects of catechin degradation products will become even more apparent in the future.

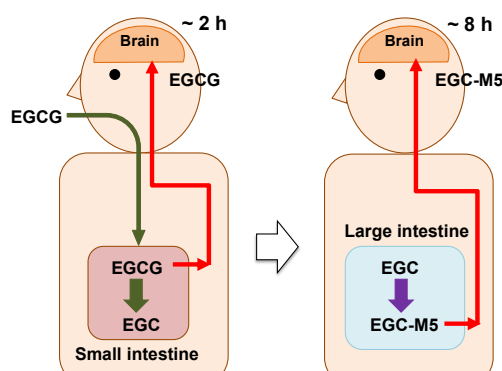


Fig. 4. A hypothesis in which EGCG and its degradation products act on the brain with a time lag

3. THEANINE AND ARGININE

Theanine is one of the amino acids that has been discovered in *Gyokuro*, a high-grade green tea. In 1950, Yajiro Sakato revealed its chemical structure to be γ -glutamylethylamide [62]. Theanine is an amino acid unique to tea. Almost no theanine is detected in other species of *Camellia* (Genus *Camellia*) plants [63]. Amino acids make up 1–8% of dried tea leaves, of which theanine makes up approximately half [64]. Since theanine is synthesized in the roots of the tea plant and stored in leaves via the trunk, it is thought that theanine is produced to store the absorbed ammonia nitrogen in a safe form [65]. The young shoots at the tip have a high content of theanine. Given that theanine is metabolized into polyphenols such as catechins in sunlight [65], blocking sunlight for two to three weeks before harvesting suppresses the decomposition of theanine and maintains it at high levels. Arginine is the second most abundant free amino acid in tea cultivated in Japan after theanine, but its content is low in Assam and its hybrids [66].

As theanine is similar to glutamic acid, which is one of the transmitters in the brain, it was predicted to have some physiological action in the brain, so many studies have been conducted to address this. Theanine absorbed from the intestine enters the brain through the BBB [67]. Furthermore, it has been reported that theanine's effects on neurotransmitters in the brain, such as dopamine [68], suppress excitation by caffeine [69], improve memory [70], have a high affinity for the glutamine transporter [70], and enact a neurogenic effect [71]. In terms of the actions of theanine in humans, a relaxing effect [26], stress reduction [24,25], and a reduction in depression and schizophrenia [72] have been reported. On the other hand, little attention has been paid to the functionality of arginine in green tea, but it has been revealed to have an excellent stress-reducing effect, similar to that of theanine [27,28]. Next, we focus on the suppression of brain aging via the stress-reducing effects of theanine and arginine, and introduce a partially elucidated mechanism.

3.1 Adrenal Hypertrophy by Psychosocial Stress and its Suppression by Theanine

Moderate stress is thought to be necessary and beneficial, but when excessive stress is experienced for a long period of time, it causes the onset and exacerbation of various diseases, such as depression, mood disorders, cardiovascular diseases, and age-related diseases [73,74]. A confrontational housing situation, using territorial male mice, increases the psychosocial stress load in each mouse, which is similar to the stress encountered by humans (Fig. 5) [75]. When mice experience stress, stress responses, such as changes in hormone secretion and adrenal hypertrophy, are observed in the activation of the hypothalamus–pituitary–adrenal (HPA) axis via excitatory signals. Adrenal hypertrophy and the altered diurnal rhythm of glucocorticoids were observed in mice placed in confrontational housing. However, mice that had received theanine (6 mg/kg) daily for several days beforehand were normal. The common ddY mouse, which is widely used outbred strain in various experiments, was used in this experiment [75]. The normal circadian rhythm of glucocorticoids has been reported to be important for synaptogenesis in the brain [76], and stress-induced hormonal

disturbances are thought to cause cognitive decline. The normalization of the HPA axis by theanine may also affect brain function.

Adrenal hypertrophy, due to confrontational housing, has been observed in all strains of male mice examined so far, but differences in resilience to stress have been found. For example, adrenal hypertrophy began to decrease after 10 days of stress loading in ddY mice [75], but hypertrophy was observed even after 7 months in senescence-accelerated SAMP10 mice [77]. SAMP10 mice have a shorter lifespan than normal mice, and brain atrophy and decreased brain function are observed with aging [77]. Theanine showed an excellent suppressive effect on adrenal hypertrophy in mice of both strains. In females, adrenal hypertrophy was not clearly observed under confrontational housing conditions, because females are less territorial than males.

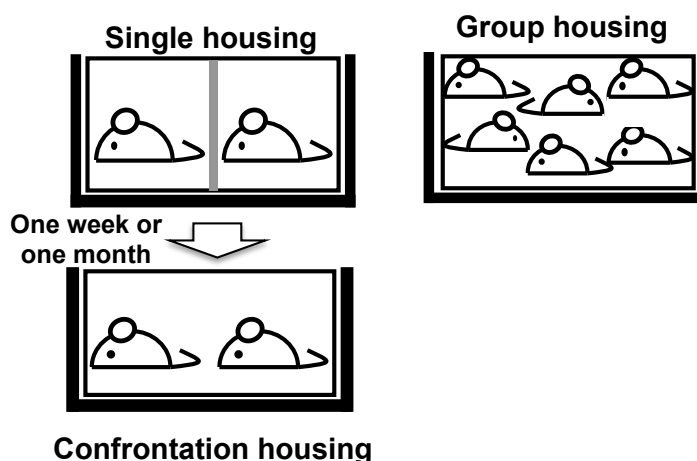


Fig. 5. Confrontation housing as a psychosocial stress loading scenario. To create confrontational housing, a standard polycarbonate cage was divided into two identical subunits with a stainless-steel partition (single housing). Two mice were housed in the partitioned cage for one week (for a short-term experiment) or one month (for a long-term experiment). The partition was then removed. Subsequently, the two mice co-inhabited the same cage (confrontational housing). Group-housed mice were housed in groups of six as a control housing scenario. These mice ingested water with or without theanine (6 mg/kg)

3.2 Longevity Shortened by Stress and its Suppression by Theanine and Arginine

The average survival time of SAMP10 mice was 17.6 ± 1.2 months under normal group housing conditions, but decreased significantly under confrontational housing conditions to 13.6 ± 1.5 months (Fig. 6) [77]. Survival was reduced by three quarters due to stress loading. However, mice that drank water containing theanine (6 mg/kg) under the same stress-loading conditions showed a similar survival time as group-housed mice (17.9 ± 1.4 months). The longevity of group-housed mice was not prolonged by theanine intake, indicating that theanine improves survival time by reducing stress. On the other hand, no shortening of lifespan due to stress load was observed in ddY mice. Individual differences in susceptibility to stress are well known, but it is not yet fully understood what causes these differences. SAMP10 and ddY mice may be suitable laboratory animals to assess differences in stress sensitivity.

Median survival time (MST) was significantly longer in SAMP10 mice that ingested arginine (3 mg/kg) under stress-loading conditions (16.6 months) than in mice that did not receive any arginine (10.5 months) [28]. The dose of arginine was based on data that showed the suppression of adrenal hypertrophy, which was observed to occur at approximately half the dose of theanine [27]. It was also determined by the fact that arginine represents approximately half the amount of theanine in green tea.

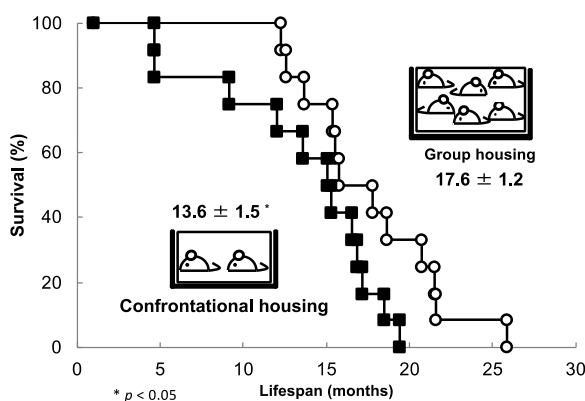


Fig. 6. The effect of confrontational housing on the lifespan of SAMP10 mice. Mice were housed alone for one month before they were introduced into confrontational housing conditions. The maximum survival time was approximately 20 months (closed squares). In contrast, group-housed control mice began to die at around 12 months of age, with a maximum survival time of 26 months (open circles) (n = 12) [77]

3.3 Promotion of Cognitive Decline Due to Stress Load, and Accumulation of Oxidative Damage

SAMP10 mice underwent a significant reduction in learning ability after 11 months of age, but no reduction was observed at 8 months of age. However, under stress-loading conditions, learning ability declined as early as 8 months of age, indicating that stress promotes a decline in brain function [77]. On the other hand, no reduction in brain function was observed in stress-loaded mice that ingested theanine or arginine [28,77]. Since the brain consumes a large amount of oxygen, it is susceptible to oxidative damage due to the production of many reactive oxygen species (ROS) during metabolism [78]. When the level of 8-oxodeoxyguanosine in cerebral cortical DNA was measured at 9 months of age as a marker of oxidative damage, damage increased significantly in mice under stress-loaded conditions compared with mice of the same age that were housed in a group [77]. SAMP10 mice produce more ROS in the brain from a young age than normal mice [79], and the activity of glutathione peroxidase tended to decrease in aged mice [80]. Therefore, oxidative damage tends to accumulate with age. However, as theanine has no direct or strong antioxidant effect similar to catechins, the suppression of oxidative damage via the ingestion of theanine is considered to be indirectly caused by the balance of ROS production and elimination in the brain.

3.4 Brain Atrophy Due to Stress Load and Suppression by Theanine

Severely stressed people [30–32] and abused children [33–35] experience brain atrophy. SAMP10 mice show cerebral atrophy with aging, amplified by stress load [77]. In order to clarify when and where stress-induced brain atrophy occurs, the brains of mice under confrontational housing conditions were closely analyzed using ex vivo magnetic resonance scanning [81]. The mice were kept under confrontational housing conditions. The theanine group freely drank water containing theanine (6 mg/kg/day) while control mice drank water. Significant atrophy occurred in the cerebral cortex one month after stress loading, and then atrophy progressed in the control mice (Figure 7a) [81]. Similar levels of atrophy were observed in mice of the theanine group one month later under stress loading, but these recovered after 2 months. The hippocampus tended to become atrophic one month after the start of stress loading, but at 6 months, the hippocampus of mice in the theanine group was significantly larger than that of control mice [81]. The cerebral cortex of ddY mice became atrophic in the control group one month after the start of stress loading but recovered thereafter [81] (Figure 7b). No atrophy was observed in cerebral cortex of ddY mice in the theanine group. Similar effects were observed in the hippocampus. There were differences in stress-induced brain atrophy between SAMP10 and ddY mice. These results confirm that stress-induced atrophy of the brain

occurs at an early stage, and that theanine is involved in the suppression and recovery of stress-induced brain atrophy.

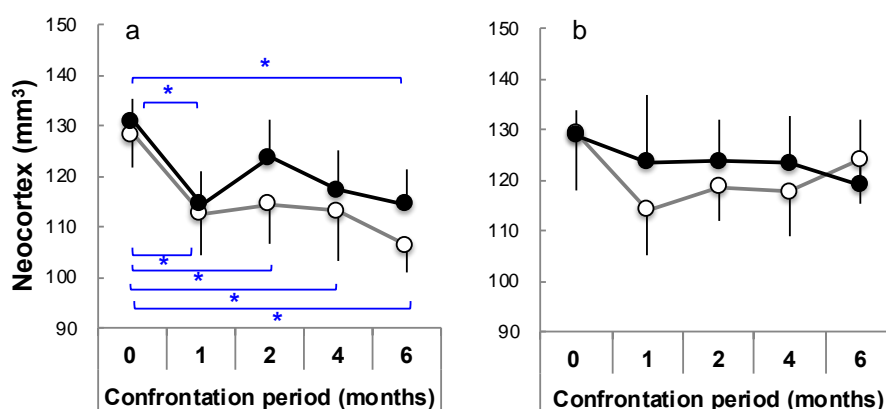


Fig. 7. Time course of the neocortex volume in SAMP10 (a) and ddY mice (b) between the control (open circles) and theanine (closed circles) groups under confrontational housing conditions. The mice were dissected after 0, 1, 2, 4, and 6 months of confrontational housing and subjected to measurements of ex vivo magnetic resonance scanning (n = 3–12; *, p < 0.05) [81]

3.5 Changes in Hippocampal Gene Expression Due to Stress Loading and Suppression by Theanine or Arginine

Since changes in the initial stage of stress loading were clearly important, changes in hippocampal gene expression on the third day of stress loading were comprehensively investigated. The expression of lipocalin 2 (*Lcn2*) markedly increased in SAMP10 mice after stress loading but was clearly suppressed following theanine intake [81] (Figure 8). The increase in ddY was slight. Excessive *Lcn2* expression has been reported to lead to increased neuronal cell death, as *Lcn2* is released from activated astrocytes and increases nerve cell damage and inflammation [82]. Excessive *Lcn2* expression in SAMP10 mice is thought to be a primary cause of brain atrophy. In addition, the control of *Lcn2* by theanine may be critical in suppressing the chronic inflammation associated with aging and neurodegenerative diseases.

On the other hand, the expression of *Npas4* was significantly reduced in the hippocampus of SAMP10 mice by stress loading, but this reduction was suppressed in mice that ingested theanine [81] (Figure 8). In ddY mice, the expression of *Npas4* increased, but was slightly suppressed in the theanine group. *Npas4*, an IEG, plays an important role in neural activity-dependent synaptogenesis as a transcription factor and is closely involved in anxiety, depressive behavior, and learning behavior [83]. It has been reported that *Npas4* expression in the hippocampus decreases when rats are subjected to chronic stress, but this level is more effectively restored in stress-resistant (resilient) compared to stress-vulnerable rats [84]. These early changes in gene expression are considered to be one reason for the stress vulnerability of SAMP10 mice. SAMP10 is vulnerable to stress, but theanine may enhance resilience by regulating the expression of *Npas4* and *Lcn2*.

Glutamate, a major excitatory neurotransmitter, regulates the HPA axis via glutamate receptors in the paraventricular nucleus of the hypothalamus [85]. Theanine, taken into the brain, acts strongly on the glutamine transporter and suppresses hyperexcitability by suppressing the supply of glutamate [70]. Since *Npas4* also regulates nerve excitement and inhibition, it is necessary to investigate how the balance between glutamate and γ -aminobutyric acid (GABA), an inhibitory neurotransmitter, is altered by theanine intake.

Next, to investigate the mechanism of the anti-stress effect of arginine, early changes in gene expression levels were measured in SAMP10 mice on day three of stress loading [28]. The genes that

maintain mitochondrial functions and neuronal survival, including hemoglobin alpha, adult chain 2 (*Hba-a2*), and hemoglobin beta, adult minor chain (*Hbb-b2*), increased significantly in mice that ingested arginine. In contrast, several genes associated with the oxidative stress response and neuronal excitotoxic cell death, including nuclear receptor subfamily 4, group A, member 1 (*Nr4a1*), activity-regulated cytoskeleton-associated protein (*Arc*), and cysteine-rich protein 61 (*Cyr61*), increased remarkably in response to stress. However, their expression was significantly suppressed in mice that ingested arginine. In fact, lipid peroxidation in the brains of stressed mice was significantly lowered by arginine intake [28]. These results indicate that arginine reduces oxidative damage and enhances mitochondrial functions in the brain.

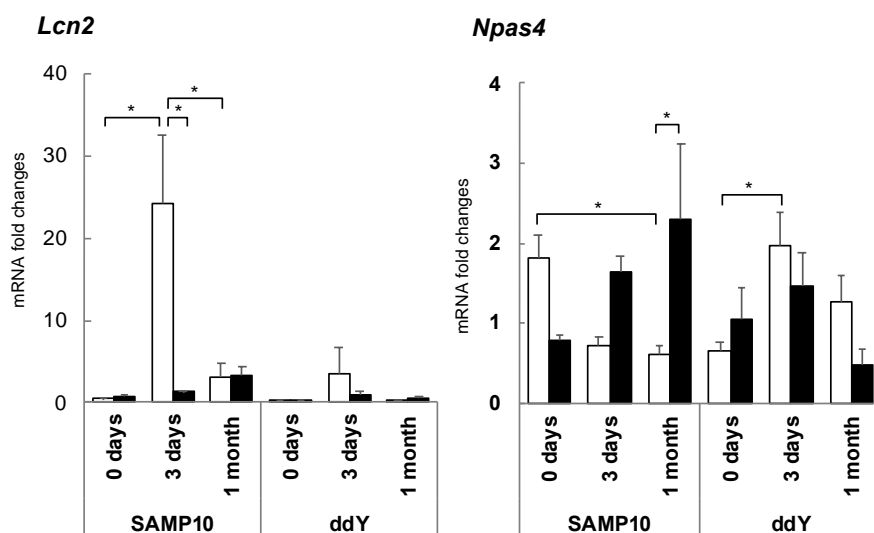


Fig. 8. Expression levels of *Lcn2* and *Npas4* mRNA in the hippocampus of SAMP10 and ddY mice. Mice consumed theanine (closed bars) or normal tap water (control, open bars) *ad libitum*. After single housing for one month, hippocampal samples were obtained from mice that were housed confrontationally after 0 days, 3 days, and 1 month. Values are expressed as the mean \pm SEM ($n = 3-6$, * $p < 0.05$) [81].

Both theanine and arginine have a stress-reducing effect, but there are differences in the expression of major genes that they each target. Since many changes occur in organisms in response to stress loading, it is possible that there are various targets in stress reduction.

3.6 Changes in Hippocampal Metabolites and Behavior One Month after Stress Loading

Furthermore, the effects of theanine intake on behavior were compared at one month after stress loading, at which point cerebral atrophy was markedly observed. As a result, in SAMP10 and ddY mice, the immobility time in the tail suspension test was significantly shortened in the theanine intake group, suggesting that theanine intake improves depressive behavior in stress-loaded mice [86]. After this, changes in metabolites in the hippocampus were investigated. The hippocampus is a stress-sensitive tissue in the brain. For example, chronic stress has been shown to significantly reduce hippocampal volume in mice and impair hippocampal neurogenesis [87]. Neurogenesis in the hippocampus occurs throughout life, which regulates the inhibitory circuitry [88] and could be associated with hippocampus-dependent learning and memory [89–91].

Among the statistically altered metabolites, kynurenine (Kyn) and histamine were significantly higher in SAMP10 mice than in ddY mice. Kyn increased in chronically stressed rats [92]. In addition, the Kyn pathway plays a key role in depressive behavior in mice [93,94]. Since Kyn is produced from tryptophan via indoleamine-2,3-dioxygenase (IDO), the high expression level of IDO in SAMP10 mice under stress loading may contribute to the high level of Kyn in SAMP10 mice [86]. Histamine, which is

enhanced by a variety of stressors, has strong effects on excitability in the hippocampus [95]. The synthesis of histamine is controlled by the inhibitory H3 auto-receptors located on histamine neurons [96]. Theanine intake significantly suppresses histamine levels in stress-loaded SAMP10 mice [86], suggesting that the histaminergic system is an important target for theanine. In addition, theanine has been suggested to improve sleep quality [72,97]. It is interesting that histamine likely plays a pivotal role in the regulation of sleep–wakefulness via H1 and/or H3 receptors [96].

On the other hand, carnosine has been shown to be present at high concentrations in the brain and has been reported to have antidepressant effects [98,99]. Carnosine levels were significantly lower in SAMP10 mice than in ddY mice, but these levels were significantly increased in SAMP10 mice that ingested theanine under confrontational housing conditions [86]. In addition, the levels of ornithine, which has anti-stress effects, were significantly lower in SAMP10 mice than in ddY mice [86].

In summary, depressive behavior was observed under long-term stress conditions. Here, depression-related Kyn and excitement-related histamine levels increased in the hippocampus, while antidepressant-related carnosine and anti-stress-related ornithine levels decreased. It has been suggested that theanine intake regulates their levels and improves depressive behavior. These metabolic differences may help to determine stress vulnerability.

3.7 Brain Aging Acceleration Due to Psychosocial Stress

The relationship between stress and brain aging described in this section can be summarized as follows (Fig. 9). In stress-vulnerable mice (SAMP10), brain atrophy was observed at least one month after stress loading, which then further progressed with aging, resulting in aging promotion (decreased brain function and shortened lifespan). On the other hand, in stress-resilient mice (ddY), the degree of atrophy in the brain due to the stress load was not significant. Furthermore, this atrophy was recovered, and no effect on brain function or longevity was observed.

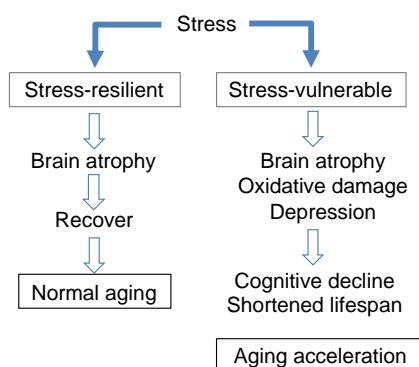


Fig. 9. The relationship between stress and brain aging.

In SAMP10 mice given theanine, stress-induced atrophy in the brain subsequently recovered, but not completely. No atrophy was observed in ddY mice that received theanine. In addition, mice that ingested theanine and arginine exhibited suppressed cognitive decline and a shortened lifespan. Stress seems to cause neuroinflammation, oxidative stress, imbalances in excitation/suppression, and the suppression of neurogenesis in the brain. Although some of the genes targeted by theanine and arginine have been identified in the early stages of stress loading, further research is needed to elucidate the effects of these two compounds on complex biological stress responses. Nevertheless, for those who are vulnerable to stress, theanine and arginine are probably essential substances.

4. FUNCTION OF GREEN TEA

Green tea (*Camellia sinensis* (L.) O. Kuntze) is an unfermented tea that remains green via the rapid inactivation of enzymes during heating, such as polyphenol oxidase, which prevent the oxidation of tea's components. There are two main types of green tea [100]. One is *Sencha*, which is made from

ordinary tea leaves and is rich in catechins. The other includes *Gyokuro* and *Tencha*, which are deprived of sunlight for approximately 20 days before harvesting. Shaded tea leaves have a high content of theanine because their metabolization into catechins is suppressed. The content of theanine is higher in higher-grade green tea. Matcha tea is made by grinding *Tencha* into a fine powder with a stone mill. Catechins, caffeine, and amino acids containing theanine provide the taste components of astringency, bitterness, and umami, respectively. In addition, the balance of these ingredients affects the functionality of the tea.

4.1 Stress-Reducing Effect of Green Tea with Lowered Caffeine

It has been determined that the stress-reducing effect of theanine is strongly suppressed by the presence of caffeine and EGCG [27]. Therefore, the amount of caffeine in picked tea leaves can be reduced to 1/3–1/4 by spraying them with hot water [27]. The tea produced in this way is called low-caffeine green tea.

The elution of caffeine and EGCG depends on the water temperature—their degree of elution decreases in cold water—but amino acids and EGC are less affected by water temperature [101]. Therefore, low-caffeine green tea eluted with cold water has low levels of caffeine and EGCG and relatively high levels of theanine, arginine, and EGC. The mean value of each component of green tea used in clinical studies with participants in their 80s–90s is shown in Table 2.

Table 2. The content of caffeine, catechins, and amino acids in a solution of green tea

Green tea	Caffeine (mg/L)	Catechins (mg/L)						Total		
		EGCG	EGC	ECG	EC	CG	(+) C			
Standard	120	117	73.2	26.5	43.9	0.41	5.47	266		
Low-caffeine	37.2	64.7	145	10.7	53.8	0.26	4.13	278		
Green tea	Theanine	Free amino acids (mg/L)								
		Glu	Arg	Asp	Gln	Ser	Ala	Asn	GABA	Total
Standard	36.0	9.12	7.28	7.44	5.34	2.99	0.90	0.76	0.47	70.4
Low-caffeine	85.2	18.2	21.6	12.9	15.8	5.73	2.16	1.21	1.51	164

Low-caffeine green tea (20 g) was steeped in 2000 mL of room-temperature water. Standard green tea (10 g) was steeped in 2000 mL of boiling water. EGCG, (-) epigallocatechin gallate; EGC, (-) epigallocatechin; ECG, (-) epicatechin gallate; EC, (-) epicatechin; CG, (-) catechin gallate; (+) C, (+) catechin; Glu, glutamic acid; Arg, arginine; Asp, aspartic acid; Gln, glutamine; Ser, serine; Ala, alanine; Asn, asparagine; GABA, γ -amino butyric acid

Participants in their 20s (young), 40s–50s (middle-aged), and 80s–90s (elderly) drank low-caffeine green tea eluted with room-temperature water. Salivary alpha-amylase is a non-invasive biomarker of the sympathetic nervous system [102]. The activity of salivary amylase, which was assessed in subjects immediately after they had woken up in the morning, in the low-caffeine green tea intake group were lower than in the control group (standard green tea or barley tea) in all age groups [103–105]. This indicates that the ingestion of low-caffeine green tea is useful for suppressing stress. A self-diagnostic assessment of accumulated fatigue and work severity, which was created by the Japanese Ministry of Health, Labor, and Welfare, was carried out every Monday morning and every Friday evening throughout the test period. The degree of fatigue accumulation on Monday morning was significantly lower in the middle-aged low-caffeine green tea intake group [104]. An electroencephalogram (EEG) was taken during sleep using a single-channel EEG (Sleep Scope, SleepWell Co., Osaka, Japan). Although there were individual differences in sleep characteristics, early-morning awakening was suppressed in the elderly low-caffeine green tea intake group [105]. A similar effect was observed in middle-aged participants [104]. Early-morning awakening, with an inability to return to sleep, is a common sleep problem in older people [106], and so the intake of low-caffeine green tea may improve their quality of sleep. In addition, in elderly participants whose salivary amylase activity was lowered by the ingestion of low-caffeine green tea, several sleep parameters, such as sleep efficiency, sleep onset latency, and non-REM sleep, improved [105]. These data show that reducing the levels of caffeine and EGCG in green tea not only enhances the stress-reducing effects of theanine but also improves sleep. Better sleep is broadly beneficial because chronic

insomnia can increase the risk of cognitive impairment, anxiety, depression, and cardiovascular disease [107].

4.2 Stress Reduction by Matcha

Matcha is a green tea that contains more theanine and caffeine, and fewer catechins, than *Sencha*. However, these components vary considerably depending on the cultivation conditions and harvest time. Therefore, some samples of *Matcha* were evaluated for their stress-reducing effects via an animal experiment. Here, *Matcha* with a CE/TA ratio of 2 or less—where the CE/TA ratio indicates the molar ratio of the sum of caffeine (C) and EGCG (E) to the sum of theanine (T) and arginine (A)—was able to suppress stress, indicated by adrenal hypertrophy, in mice [29].

The stress-reducing effect of *Matcha* suspended in water was examined in participants in their 20s. When evaluated by salivary amylase activity, *Matcha* with a CE/TA ratio of 1.79 was shown to reduce stress, but that with a CE/TA ratio of 10.79 did not show any effect [29]. A similar difference in effect was observed when these *Matcha* varieties were baked into cookies and ingested [108].

Of the 76 brands of *Matcha* sold in Japan, 32 had a CE/TA ratio of 2 or less [29] (Fig. 10). Of the 67 brands of *Matcha* sold outside of Japan, only one brand had a CE/TA ratio of 2 or less. These data indicate that not all *Matcha* varieties can be expected to have a stress-relieving effect.

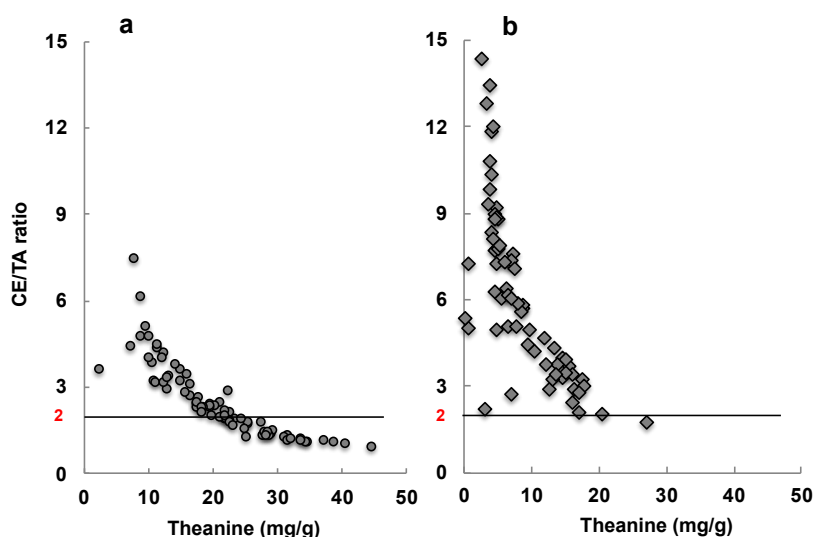


Fig. 10. The amount of theanine, and the molar ratio of caffeine and EGCG to theanine and arginine, in 76 samples of *Matcha* marketed in Japan (a) and 67 samples of *Matcha* marketed outside of Japan (b)

4.3 Anti-Depressant Effect of Shaded White Leaf Tea

When a green tea plant is completely shaded for approximately two weeks, its levels of amino acids can be increased six- to seven-fold relative to ordinary *Sencha*. The green tea produced in this way is called shaded white leaf tea (SWLT), and it is gaining interest as a green tea with a strong umami taste [109]. In SWLT, the level of theanine is about 5-fold, and the level of arginine is about 13-fold, higher than normal *Sencha* (Table 3). The CE/TA ratio of SWLT is 1.12, suggesting a stress-reducing effect. However, no significant stress-reducing effect of this variety was observed in a clinical study [110].

In SWLT, complete shading for two weeks causes the decomposition of soluble proteins, and its free amino acid composition was significantly different from that of general *Sencha* [110]. Although theanine levels were higher in this variety, the proportion of theanine in its total amino acid level was

lower than in *Sencha*, while the proportions of arginine, glutamine, asparagine, aspartic acid, etc., were higher. An increase in asparagine and aspartic acid was found to suppress the anti-stress effect of theanine [110]. Furthermore, no stress-reducing effect was observed in the eluate with a CE/TA ratio of 0.9 or higher. It seems that tea eluate and *Matcha* are somewhat different.

Table 3. The caffeine, catechin, and amino acid contents in the eluate of SWLT and *Sencha*

Tea	Caffeine (mg/L)	Catechins (mg/L)						
		EGCG	EGC	ECG	EC	GC	CG	(+) C
SWLT	209.8	150.4	135.2	24.6	41.0	5.0	2.8	3.4
<i>Sencha</i>	112.0	134.2	229.0	21.0	46.6	13.6	4.6	2.0

Tea	Free amino acids (mg/L)								
	Theanine	Arg	Gln	Asn	Asp	Glu	Ser	GABA	Total
SWLT	140.2	69.9	51.7	33.8	33.5	19.3	12.6	0	361.0
<i>Sencha</i>	28.8	5.4	3.9	0.7	5.5	6.9	2.2	0	53.5

Shaded white leaf tea (SWLT) and Sencha green tea (3 g) were steeped in 500 mL room-temperature water for 3 h. EGCG, (-) epigallocatechin gallate; EGC, (-) epigallocatechin; ECG, (-) epicatechin gallate; EC, (-) epicatechin; GC, (-) gallic catechin; CG, (-) catechin gallate; (+) C, (+) catechin; Arg, arginine; Gln, glutamine; Asn, asparagine; Asp, aspartic acid; Glu, glutamic acid; Ser, serine; GABA, γ -amino butyric acid

On the other hand, depressive behavior was significantly suppressed in mice that ingested SWLT for one month [110]. Depression is the most common mental illness, and stress is a major risk factor for it. Stress loads initially cause excitement, but chronic stress leads to depression. Therefore, in the context of long-term stress, a green tea that not only reduces stress but also alleviates depression is necessary. The intake of green tea has been reported to prevent depression, suggesting the involvement of caffeine and catechins [111]. For example, caffeine and EGCG have been reported to increase the expression of glutamate, a major excitatory neurotransmitter in the brain, while inhibiting GABA, an inhibitory neurotransmitter [112,113]. Balancing excitement and depression in the brain is considered to be very important for maintaining physical and mental health. In the near future, it will be possible to produce green tea with a CE/TA ratio suitable for improving depression in humans.

5. CONCLUSIONS

Epidemiological and animal studies have suggested that daily intake of green tea catechins suppresses age-related cognitive decline. EGCG, the main catechin in green tea, has been suggested to activate nerve cells, and its metabolic decomposition products act similarly, but there is a time lag. Based on the BBB permeability of catechins and their degradation products, several cups of green tea, or approximately one bottle (500 mL) of catechins, may reduce the age-related decline in cognitive function.

In addition, theanine and arginine were shown to have an excellent stress-reducing effect and to suppress the shortening of lifespan and the deterioration in cognitive function due to stress. Since brain atrophy followed by aging promotion, due to psychosocial stress, were observed in stress-vulnerable mice, but not in stress-resistant ones, theanine and arginine are probably essential substances for individuals who are vulnerable to stress.

However, the effect of theanine and arginine was shown to be greatly determined by the simultaneous presence of caffeine and EGCG. Differences in the molar ratio of CE/TA (caffeine + EGCG/theanine + arginine) in green tea were shown to affect stress reduction and sleep in experimental and clinical studies.

EGCG and theanine, unique ingredients in green tea, affect brain function. The daily intake of a quantity of green tea appropriate for each physical and mental health condition is suggested to suppress brain aging by activating nerve cells and reducing stress.

AUTHOR CONTRIBUTIONS

Writing—original draft preparation, author KU. writing—review and editing, author YN. Both authors have read and agreed to the published version of the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nishizaki Y, Kuwahira I, Kawada H, Kubo A, Kataoka K, Tanaka S, et al. Beneficial effects of medical advice provided to elderly persons under the anti-aging health check-up system at Tokai University Tokyo Hospital. *Tokai J. Exp. Clin. Med.* 2009;34:142–151.
2. Saito E, Inoue M, Sawada N, Shimazu T, Yamaji T, Iwasaki M, et al. JPHC Study Group. Association of green tea consumption with mortality due to all causes and major causes of death in a Japanese population: The Japan Public Health Center-based Prospective Study (JPHC Study). *Ann. Epidemiol.* 2015;25:512–518.e3.
DOI:10.1016/j.annepidem.2015.03.007.
3. Noguchi-Shinohara M, Yuki S, Dohmoto C, Ikeda Y, Samuraki M, Iwasa K, et al. Consumption of green tea, but not black tea or coffee, is associated with reduced risk of cognitive decline. *PLoS ONE.* 2014;9:e96013.
DOI:10.1371/journal.pone.0096013.
4. Kakutani S, Watanabe H, Murayama N. Green Tea Intake and Risks for Dementia, Alzheimer's Disease, Mild Cognitive Impairment, and Cognitive Impairment: A Systematic Review. *Nutrients* 2019, 11, 1165,
DOI:10.3390/nu11051165.
5. Ran LS, Liu WH, Fang YY, Xu SB, Li J, Luo X, Pan DJ, Alcohol, coffee and tea intake and the risk of cognitive deficits: A dose-response meta-analysis. *Epidemiol. Psychiatr. Sci.* 2021, 30, e13,
DOI:10.1017/S2045796020001183.
6. Zhang J, Wang A, Zhang X, Chen S, Wu S, Zhao X, Zhang Q. Association between tea consumption and cognitive impairment in middle-aged and older adults. *BMC Geriatr.* 2020;20: 447,
DOI:10.1186/s12877-020-01848-6.
7. Okubo H, Inagaki H, Gondo Y, Kamide K, Ikebe K, Masui Y, et al. Association between dietary patterns and cognitive function among 70-year-old Japanese elderly: A cross-sectional analysis of the SONIC study. *Nutr. J.* 2017;16:56.
DOI:10.1186/s12937-017-0273-2.
8. Pervin M, Unno K, Ohishi T, Tanabe H, Miyoshi N, Nakamura Y. Beneficial Effects of Green Tea Catechins on Neurodegenerative Diseases. *Molecules.* 2018;23:1297.
DOI:103390/molecules23061297.
9. Singh NA, Mandal AK, Khan ZA. Potential neuroprotective properties of epigallocatechin-3-gallate (EGCG). *Nutr. J.* 2016;15:60.
DOI:101186/s12937-016-0179-4.
10. Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeanniton D, Ehrhart J, et al. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J. Neurosci.* 2005;25:8807–8814.
DOI:10.1523/JNEUROSCI.1521-05.2005.
11. Mandel SA, Amit T, Kalfon L, Reznichenko L, Weinreb O, Youdim MB. Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: Special reference to epigallocatechin gallate (EGCG). *J. Alzheimers Dis.* 2008;15:211–222.
DOI:10.3233/jad-2008-15207.
12. Mithul Aravind S, Wichienchot S, Tsao R, Ramakrishnan S, Chakkaravarthi S. Role of dietary polyphenols on gut microbiota, their metabolites and health benefits. *Food Res. Int.* 2021;142: 110189.
DOI:101016/j.foodres.2021.110189.
13. Clifford MN, van der Hoof JJ, Crozier A. Human studies on the absorption, distribution, metabolism, and excretion of tea polyphenols. *Am. J. Clin. Nutr.* 2013;98(Suppl. 6):1619S–1630S,
DOI:103945/ajcn.113.058958.

14. Liu Z, Bruins ME, Ni L, Vincken JP. Green and Black Tea Phenolics: Bioavailability, Transformation by Colonic Microbiota, and Modulation of Colonic Microbiota. *J. Agric. Food Chem.* 2018;66:8469–8477.
DOI:10.1021/acs.jafc.8b02233.
15. Angelino D, Carregosa D, Domenech-Coca C, Savi M, Figueira I, Brindani N, et al. 5-(Hydroxyphenyl)- γ -Valerolactone-Sulfate, a Key Microbial Metabolite of Flavan-3-ols, Is Able to Reach the Brain: Evidence from Different *In Silico*, *In Vitro* and *In Vivo* Experimental Models. *Nutrients.* 2019;11:2678.
DOI:10.3390/nu11112678.
16. Pervin M, Unno K, Takagaki A, Isemura M, Nakamura Y. Function of Green Tea Catechins in the Brain: Epigallocatechin Gallate and its Metabolites. *Int. J. Mol. Sci.* 2019;20:3630.
DOI:10.3390/ijms20153630.
17. Unno K, Pervin M, Nakagawa A, Iguchi K, Hara A, Takagaki A, Nanjo F, Minami A, Nakamura Y. Blood–brain Barrier Permeability of Green Tea Catechin Metabolites and their Neurotogenic Activity in Human Neuroblastoma SH-SY5Y Cells. *Mol. Nutr. Food Res.* 2017;61:e1700294.
DOI:10.1002/mnfr.201700294.
18. Takagaki A, Yoshioka Y, Yamashita Y, Nagano T, Ikeda M, Hara-Terawaki A, Seto R, Ashida H. Effects of Microbial Metabolites of (-)-Epigallocatechin Gallate on Glucose Uptake in L6 Skeletal Muscle Cell and Glucose Tolerance in ICR Mice. *Biol. Pharm. Bull.* 2019;42:212–221.
DOI:10.1248/bpb.b18-00612.
19. Kim YH, Won YS, Yang X, Kumazoe M, Yamashita S, Hara A, Takagaki A, Goto K, Nanjo F, Tachibana H. Green Tea Catechin Metabolites Exert Immunoregulatory Effects on CD4(+) T Cell and Natural Killer Cell Activities. *J. Agric. Food Chem.* 2016;64:3591–3597.
DOI:10.1021/acs.jafc.6b01115.
20. Takagaki A, Otani S, Nanjo F. Antioxidative activity of microbial metabolites of (-)-epigallocatechin gallate produced in rat intestines. *Biosci. Biotechnol. Biochem.* 2011;75:582–585.
DOI:10.1271/bbb.100683.
21. Unno T, Tamemoto K, Yayabe F, Kakuda T. Urinary excretion of 5-(3',4'-dihydroxyphenyl)- γ -valerolactone, a ringfission metabolite of (-)-epicatechin, in rats and its *in vitro* antioxidant activity. *J. Agric. Food Chem.* 2003;51:6893–6898.
DOI:10.1021/jf034578e.
22. Williams JL, Everett JM, D'Cunha NM, Sergi D, Georgousopoulou EN, Keegan RJ, et al. The Effects of Green Tea Amino Acid L-Theanine Consumption on the Ability to Manage Stress and Anxiety Levels: A Systematic Review. *Plant Foods Hum. Nutr.* 2020;75:12–23.
DOI:10.1007/s11130-019-00771-5.
23. Hidese S, Ogawa S, Ota M, Ishida I, Yasukawa Z, Ozeki M, Kunugi H. Effects of L-Theanine Administration on Stress-Related Symptoms and Cognitive Functions in Healthy Adults: A Randomized Controlled Trial. *Nutrients.* 2019;11:2362.
DOI:10.3390/nu11102362.
24. Unno K, Tanida N, Ishii N, Yamamoto H, Iguchi K, Hoshino M, et al. Anti-stress effect of theanine on students during pharmacy practice: Positive correlation among salivary α -amylase activity, trait anxiety and subjective stress. *Pharmacol. Biochem. Behav.* 2013;111:128–135.
DOI:10.1016/j.pbb.2013.09.004.
25. Kimura K, Ozeki M, Juneja LR, Ohira H. L-Theanine reduces psychological and physiological stress responses. *Biol. Psychol.* 2007;74:39–45.
DOI:10.1016/j.biopsycho.2006.06.006.
26. Kobayashi K, Nagato Y, Aoi N, Juneja LR, Kim M, Yamamoto T, Sugimoto S. Effect of L-theanine on the release of α -brain wave in human volunteers. *Nippon Nogeikagaku Kaishi.* 1998;72:153–157.
27. Unno K, Hara A, Nakagawa A, Iguchi K, Ohshio M, Morita A, Nakamura Y. 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.* 2016; 23:1365–1374,
DOI:10.1016/j.phymed.2016.07.006.

28. Pervin M, Unno K, Konishi T, Nakamura Y. L-Arginine Exerts Excellent Anti-Stress Effects on Stress-Induced Shortened Lifespan, Cognitive Decline and Depression. *Int. J. Mol. Sci.* 2021;22:508.
DOI:103390/ijms22020508.
29. Unno K, Furushima D, Hamamoto S, Iguchi K, Yamada H, Morita A, Stress-Reducing Function of Matcha Green Tea in Animal Experiments and Clinical Trials. *Nutrients* 2018, 10, 1468,
DOI:10.3390/nu10101468.
30. Ansell EB, Rando K, Tuit K, Guarnaccia J, Sinha R. Cumulative adversity and smaller gray matter volume in medial prefrontal, anterior cingulate, and insula regions. *Biol. Psychiatry.* 2012;72:57–64.
DOI:10.1016/j.biopsych.2011.11.022.
31. Bremner JD. Stress and brain atrophy. *CNS Neurol. Disord. Drug Targets.* 2006;5:503–512.
DOI:102174/187152706778559309.
32. Schoenfeld TJ, McCausland HC, Morris HD, Padmanaban V, Cameron HA. Stress and Loss of Adult Neurogenesis Differentially Reduce Hippocampal Volume. *Biol. Psychiatry.* 2017;82:914–923,
DOI:101016/j.biopsych.2017.05.013.
33. Tomoda A, Polcari A, Anderson CM, Teicher MH. Reduced visual cortex gray matter volume and thickness in young adults who witnessed domestic violence during childhood. *PLoS ONE.* 2012;7:e52528.
DOI:10.1371/journal.pone.0052528.
34. Tomoda A, Sheu YS, Rabi K, Suzuki H, Navalta CP, Polcari A, Teicher MH. Exposure to parental verbal abuse is associated with increased gray matter volume in superior temporal gyrus. *Neuroimage.* 2011;54 (Suppl. 1):S280–S286.
DOI:101016/j.neuroimage.2010.05.027.
35. Tomoda A, Navalta CP, Polcari A, Sadato N, Teicher MH. Childhood sexual abuse is associated with reduced gray matter volume in visual cortex of young women. *Biol. Psychiatry.* 2009;66:642–648.
DOI:10.1016/j.biopsych.2009.04.021.
36. Guessoum SB, Lachal J, Radjack R, Carretier E, Minassian S, Benoit L, Moro MR. Adolescent psychiatric disorders during the COVID-19 pandemic and lockdown. *Psychiatry Res.* 2020;291:113264,
DOI:10.1016/j.psychres.2020.113264.
37. Heitzman, J. Impact of COVID-19 pandemic on mental health. *Psychiatr. Pol.* 2020;54:187–198.
DOI:1012740/PP/120373.
38. Tsujimura M. On Tea Catechin Isolated from Green Tea. *J. Agric. Chem. Soc. Jpn.* 1930;6:62–69.
39. Lopez T, Schriener SE, Okoro M, Lu D, Chiang BT, Huey J, Jafari M. Green tea polyphenols extend the lifespan of male drosophila melanogaster while impairing reproductive fitness. *J. Med. Food.* 2014;17:1314–1321,
DOI:10.1089/jmf.2013.0190.
40. Abbas S, Wink M. Epigallocatechin gallate from green tea (*Camellia sinensis*) increases lifespan and stress resistance in *Caenorhabditis elegans*. *Planta Med.* 2009;75:216–221.
DOI:10.1055/s-0028-1088378.
41. Unno K, Pervin M, Taguchi K, Konishi T, Nakamura Y. Green Tea Catechins Trigger Immediate-Early Genes in the Hippocampus and Prevent Cognitive Decline and Lifespan Shortening. *Molecules.* 2020;25:1484.
DOI:10.3390/molecules25071484.
42. Qiu L, Sautter J, Gu D. Associations between frequency of tea consumption and health and mortality: Evidence from old Chinese. *Br. J. Nutr.* 2012;108:1686–1697.
DOI:10.1017/S0007114511007173.
43. Shirai Y, Kuriki K, Otsuka R, Kato Y, Nishita Y, Tange C, Tomida M, Imai T, et al. Green tea and coffee intake and risk of cognitive decline in older adults: The National Institute for Longevity Sciences, Longitudinal Study of Aging. *Public Health Nutr.* 2020;23:1049–1057.
DOI:10.1017/S1368980019002659.

44. Kuriyama S, Hozawa A, Ohmori K, Shimazu T, Matsui T, Ebihara S, et al. Green tea consumption and cognitive function: A cross-sectional study from the Tsurugaya Project 1. *Am. J. Clin. Nutr.* 2006;83:355–361.
DOI:10.1093/ajcn/83.2.355.
45. Unno K, Takabayashi F, Kishido T, Oku N. Suppressive effect of green tea catechins on morphologic and functional regression of the brain in aged mice with accelerated senescence (SAMP10). *Exp. Gerontol.* 2004;39:1027–1034.
DOI:10.1016/j.exger.2004.03.033.
46. Unno K, Takabayashi F, Yoshida H, Choba D, Fukutomi R, Kikunaga N, et al. Daily consumption of green tea catechin delays memory regression in aged mice. *Biogerontology.* 2007;8:89–95.
DOI:10.1007/s10522-006-9036-8.
47. Unno K, Ishikawa Y, Takabayashi F, Sasaki T, Takamori N, Iguchi K, Hoshino M. Daily ingestion of green tea catechins from adulthood suppressed brain dysfunction in aged mice. *Biofactors.* 2008;34:263–271.
DOI:10.3233/BIO-2009-1080.
48. Pervin M, Unno K, Nakagawa A, Takahashi Y, Iguchi K, Yamamoto H, et al. Blood brain barrier permeability of (-)-epigallocatechin gallate, its proliferation-enhancing activity of human neuroblastoma SH-SY5Y cells, and its preventive effect on age-related cognitive dysfunction in mice. *Biochem. Biophys. Rep.* 2017;9: 180–186.
DOI:10.1016/j.bbrep.2016.12.012.
49. Chen L, Lee MJ, Li H, Yang CS. Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metab. Dispos.* 1997;25:1045–1050.
50. Nakagawa K, Okuda S, Miyazawa T. Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human plasma. *Biosci. Biotechnol. Biochem.* 1997;61:1981–1985,
DOI:10.1271/bbb.61.1981.
51. Kohri T, Matsumoto N, Yamakawa M, Suzuki M, Nanjo F, Hara Y, Oku N. Metabolic fate of (-)-[4-(3)H]epigallocatechin gallate in rats after oral administration. *J. Agric. Food Chem.* 2001;49:4102–4112,
DOI:10.1021/jf001491+.
52. Mena P, Bresciani L, Brindani N, Ludwig IA, Pereira-Caro G, Angelino D, Llorach R, et al. Phenyl- γ -valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: Synthesis, analysis, bioavailability, and bioactivity. *Nat. Prod. Rep.* 2019;36:714–752,
DOI:10.1039/c8np00062j.
53. Faria A, Pestana D, Teixeira D, Couraud PO, Romer I, Weksler B, et al. Insights into the putative catechin and epicatechin transport across blood–brain barrier. *Food Funct.* 2011;2:39–44.
DOI:10.1039/c0fo00100g.
54. Heroux NA, Osborne BF, Miller LA, Kawan M, Buban KN, Rosen JB, Stanton ME. Differential expression of the immediate early genes c-Fos, Arc, Egr-1, and Npas4 during long-term memory formation in the context preexposure facilitation effect (CPFE). *Neurobiol. Learn. Mem.* 2018;147:128–138,
DOI:10.1016/j.nlm.2017.11.016.
55. Chen Y, Wang Y, Ertürk A, Kallop D, Jiang Z, Weimer RM, Kaminker J, Sheng M. Activity-induced Nr4a1 regulates spine density and distribution pattern of excitatory synapses in pyramidal neurons. *Neuron.* 2014;83:431–443.
DOI:10.1016/j.neuron.2014.05.027.
56. Kwapis JL, Alagband Y, López AJ, Long JM, Li X, Shu G, et al. HDAC3-mediated repression of the Nr4a family contributes to age-related impairments in long-term memory. *J. Neurosci.* 2019;39:4999–5009.
DOI:10.1523/JNEUROSCI.2799-18.2019.
57. Malik AR, Urbanska M, Gozdz A, Swiech LJ, Nagalski A, Perycz M, Blazejczyk M, Jaworski J. Cyr61, a matricellular protein, is needed for dendritic arborization of hippocampal neurons. *J. Biol. Chem.* 2013;288:8544–8559,
DOI:10.1074/jbc.M112.411629.

58. Miyashita T, Kubik S, Lewandowski G, Guzowski JF. Networks of neurons, networks of genes: An integrated view of memory consolidation. *Neurobiol. Learn. Mem.* 2008;89:269–284.
59. Wang JH, Cheng J, Li CR, Ye M, Ma Z, Cai F. Modulation of Ca²⁺ signals by epigallocatechin-3-gallate (EGCG) in cultured rat hippocampal neurons. *Int. J. Mol. Sci.* 2011;12:742–754. DOI:10.3390/ijms12010742.
60. Bae JH, Mun KC, Park WK, Lee SR, Suh SI, Baek WK, et al. EGCG attenuates AMPA-induced intracellular calcium increase in hippocampal neurons. *Biochem. Biophys. Res. Commun.* 2002;290:1506–1512.
61. Del Rio D, Calani L, Cordero C, Salvatore S, Pellegrini N, Brighenti F. Bioavailability and catabolism of green tea flavan-3-ols in humans. *Nutrition.* 2010;26:1110–1116. DOI:10.1016/j.nut.2009.09.021.
62. Sakato Y. Studies on the Chemical Constituents of Tea. Part III. A New Amide Theanine. *Nippon Nogeikagaku Kaishi.* 1950;23:262–267.
63. Nagata T. Study on useful tea ingredients in leaves in Camellia plants. *Bull Tea Res Station. Ministry Agric. For.* 1986;21:59–120.
64. Horie H, Kohata K. General ingredients of tea. In *Health Science of Tea. New Possibility for Physiological Function*; Muramatsu, K, Oguni, I., Isemura, M., Yamamoto-Maeda, M., Eds.; Gakkai Shuppan Center: Tokyo, Japan. 2002;26–31.
65. Konishi S. Tea plant biochemistry. In *Science of Tea*; Muramatsu, K., Ed.; Asakura Shoten: Tokyo, Japan. 1991;32–42.
66. Ikeda N, Horie H, Mukai T, Goto T. Content of Individual Free Amino Acids in the First and Autumn Flushes of Cultivars for Several Tea Types. *Tea Res. J.* 1993;78:67–75.
67. Yamada T, Terashima T, Okubo T, Juneja LR, Yokogoshi H. Effects of theanine, γ -glutamylethylamide, on neurotransmitter release and its relationship with glutamic acid neurotransmission. *Nutr. Neurosci.* 2005;8:219–226, DOI:10.1080/10284150500170799.
68. Yokogoshi H, Kobayashi M, Mochizuki M, Terashima T. Effect of theanine, γ -glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats. *Neurochem. Res.* 1998;23:667–673. DOI:10.1023/a:1022490806093.
69. Kakuda T, Nozawa A, Unno T, Okamura N, Okai O. Inhibiting effects of theanine on caffeine stimulation evaluated by EEG in the rat. *Biosci. Biotechnol. Biochem.* 2000;64:287–293. DOI:10.1271/bbb.64.287. PMID: 10737183.
70. Kakuda T. Neuroprotective effects of theanine and its preventive effects on cognitive dysfunction. *Pharmacol. Res.* 2011;64:162–168. DOI:10.1016/j.phrs.2011.03.010.
71. Yoneda Y, Kawada K, Kuramoto N. Selective Upregulation by Theanine of Slc38a1 Expression in Neural Stem Cell for Brain Wellness. *Molecules* 2020;25:347. DOI:10.3390/molecules25020347.
72. Hidese S, Ota M, Wakabayashi C, Noda T, Ozawa H, Okubo T, Kunugi H. Effects of chronic l-theanine administration in patients with major depressive disorder: An open-label study. *Acta Neuropsychiatr.* 2017;29:72–79. DOI:10.1017/neu.2016.33.
73. Juruena MF, Erer F, Cleare AJ, Young AH. The Role of Early Life Stress in HPA Axis and Anxiety. *Adv. Exp. Med. Biol.* 2020;1191:141–153. DOI:10.1007/978-981-32-9705-0_9.
74. Dar T, Radfar A, Abohashem S, Pitman RK, Tawakol A, Osborne MT. Psychosocial Stress and Cardiovascular Disease. *Curr Treat. Options Cardiovasc. Med.* 2019;21:23. DOI:10.1007/s11936-019-0724-5.
75. Unno K, Iguchi K, Tanida N, Fujitani K, Takamori N, Yamamoto H, Ishii N, et al. Ingestion of theanine, an amino acid in tea, suppresses psychosocial stress in mice. *Exp. Physiol.* 2013;98:290–303. DOI:10.1113/expphysiol.2012.065532.
76. Liston C, Cichon JM, Jeanneteau F, Jia Z, Chao MV, Gan WB. Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance. *Nat. Neurosci.* 2013;16:698–705, DOI:10.1038/nn.3387.

77. Unno K, Fujitani K, Takamori N, Takabayashi F, Maeda K, Miyazaki H, et al. Theanine intake improves the shortened lifespan, cognitive dysfunction and behavioural depression that are induced by chronic psychosocial stress in mice. *Free Radic. Res.* 2011;45:966–974, DOI:10.3109/10715762.2011.566869.
78. Taylor DL, Edwards AD, Mehmet H. Oxidative metabolism, apoptosis and perinatal brain injury. *Brain Pathol.* 1999;9:93–117, DOI:10.1111/j.1750-3639.1999.tb00213.x.
79. Sasaki T, Unno K, Tahara S, Shimada A, Chiba Y, Hoshino M, Kaneko T. Age-related increase of superoxide generation in the brains of mammals and birds. *Aging Cell.* 2008;7:459–469. DOI:10.1111/j.1474-9726.2008.00394.x.
80. Kishido T, Unno K, Yoshida H, Choba D, Fukutomi R, Asahina S, Iguchi K, Oku N, Hoshino M. 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.* 2007;8:423–430. DOI:10.1007/s10522-007-9085-7.
81. Unno K, Sumiyoshi A, Konishi T, Hayashi M, Taguchi K, Muguruma Y, et al. Theanine, the Main Amino Acid in Tea, Prevents Stress-Induced Brain Atrophy by Modifying Early Stress Responses. *Nutrients.* 2020;12:174. DOI:10.3390/nu12010174.
82. Suk K. Lipocalin-2 as a therapeutic target for brain injury: An astrocentric perspective. *Prog. Neurobiol.* 2016;144:158–172, DOI:10.1016/j.pneurobio.2016.08.001.
83. Sun X, Lin Y. Npas4: Linking Neuronal Activity to Memory. *Trends Neurosci.* 2016;39:264–275. DOI:101016/j.tins.2016.02.003.
84. Benatti C, Radighieri G, Alboni S, Blom JMC, Brunello N, Tascetta F. Modulation of neuroplasticity-related targets following stress-induced acute escape deficit. *Behav. Brain Res.* 2019;364:140–148, DOI:101016/j.bbr.2019.02.023.
85. Evanson NK, Van Hooren DC, Herman JP. GluR5-mediated glutamate signaling regulates hypothalamo-pituitary-adrenocortical stress responses at the paraventricular nucleus and median eminence. *Psychoneuroendocrinology.* 2009;34:1370–1379. DOI:10.1016/j.psyneuen.2009.04.011.
86. Unno K, Muguruma Y, Inoue K, Konishi T, Taguchi K, Hasegawa-Ishii S, Shimada A, Nakamura Y. Theanine, Antistress Amino Acid in Tea Leaves, Causes Hippocampal Metabolic Changes and Antidepressant Effects in Stress-Loaded Mice. *Int. J. Mol. Sci.* 2020;22:193. DOI:103390/ijms22010193.
87. Yun J, Koike H, Ibi D, Toth E, Mizoguchi H, Nitta A, Yoneyama M, et al. Chronic restraint stress impairs neurogenesis and hippocampus-dependent fear memory in mice: Possible involvement of a brain-specific transcription factor Npas4. *J. Neurochem.* 2010;114:1840–1851.
88. Hollands C, Tobin MK, Hsu M, Musaraca K, Yu TS, Mishra R, Kernie SG, Lazarov O. Depletion of adult neurogenesis exacerbates cognitive deficits in Alzheimer's disease by compromising hippocampal inhibition. *Mol. Neurodegener.* 2017;12:64.
89. Anacker C, Hen R. Adult hippocampal neurogenesis and cognitive flexibility—Linking memory and mood. *Nat. Rev. Neurosci.* 2017;18:335–346.
90. Gonçalves JT, Schafer ST, Gage FH. Adult neurogenesis in the hippocampus: From stem cells to behavior. *Cell.* 2016;167:897–914.
91. Lieberwirth C, Pan Y, Liu Y, Zhang Z, Wang Z. Hippocampal adult neurogenesis: Its regulation and potential role in spatial learning and memory. *Brain Res.* 2016;1644:127–140.
92. Li CC, Jiang N, Gan L, Zhao MJ, Chang Q, Liu XM, Pan RL. Peripheral and cerebral abnormalities of the tryptophan metabolism in the depressionlike rats induced by chronic unpredicted mild stress. *Neurochem. Int.* 2020;138:104771.
93. Cattelan Souza L, de Brito MLO, Jesse CR, Boeira SP, de Gomes MG, Goes ATR, Fabbro LD, Machado FR, Prigol M, Nogueira CW. Involvement of kynurenine pathway in depressive-like behaviour induced by nandrolone decanoate in mice. *Steroids* 2020;164:108727.
94. Zhang Q, Sun Y, He Z, Xu Y, Li X, Ding J, Lu M, Hu G. Kynurenine regulates NLRP2 inflammasome in astrocytes and its implications in depression. *Brain Behav. Immun.* 2020;88:471–481.

95. Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. *Prog. Neurobiol.* 2001;63:637–672.
96. Thakkar MM. Histamine in the regulation of wakefulness. *Sleep Med. Rev.* 2011;15:65–74.
97. Rao TP, Ozeki M, Juneja LR. In Search of a Safe Natural Sleep Aid. *J. Am. Coll. Nutr.* 2015;34:436–447.
98. Hipkiss AR. Possible Benefit of Dietary Carnosine towards Depressive Disorders. *Aging Dis.* 2015;6:300–303.
99. Tomonaga S, Yamane H, Onitsuka E, Yamada S, Sato M, Takahata Y, Morimatsu F, Furuse M. Carnosine-induced antidepressant-like activity in rats. *Pharmacol. Biochem. Behav.* 2008;89:627–632.
100. Nakamura Y. Characteristics of Japanese Green Tea. In *Health Benefits of Green Tea. An Evidence-Based Approach*; Hara Y, Yang CS, Isemura M, Tomita I., Eds.; CAB International: Wallingford, UK. 2017;9–18.
101. Monobe M, Ema K, Tokuda Y, Maeda-Yamamoto M. Effect on the epigallocatechin gallate/epigallocatechin ratio in a green tea (*Camellia sinensis L.*) extract of different extraction temperatures and its effect on IgA production in mice. *Biosci. Biotechnol. Biochem.* 2010;74:2501–2503.
DOI:10.1271/bbb.100498.
102. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology.* 2009;34: 486–496.
DOI:10.1016/j.psyneuen.2009.01.014.
103. Unno K, Yamada H, Iguchi K, Ishida H, Iwao Y, Morita A, Nakamura Y. Antistress Effect of Green Tea with Lowered Caffeine on Humans: A Pilot Study. *Biol. Pharm. Bull.* 2017;40:902–909.
DOI:101248/bpb.b17-00141.
104. Unno K, Noda S, Kawasaki Y, Yamada H, Morita A, Iguchi K, Nakamura Y. Reduced Stress and Improved Sleep Quality Caused by Green Tea Are Associated with a Reduced Caffeine Content. *Nutrients.* 2017;9:777.
DOI:103390/nu9070777.
105. Unno K, Noda S, Kawasaki Y, Yamada H, Morita A, Iguchi K, Nakamura Y. Ingestion of green tea with lowered caffeine improves sleep quality of the elderly via suppression of stress. *J. Clin. Biochem. Nutr.* 2017;61|:210–216.
DOI:103164/jcbrn.17-6.
106. Patel D, Steinberg J, Patel P. Insomnia in the Elderly: A Review. *J. Clin. Sleep Med.* 2018;14:1017–1024,
DOI:105664/jcsm.7172.
107. Maness DL, Khan M. Nonpharmacologic Management of Chronic Insomnia. *Am. Fam. Physician.* 2015;92:1058–1064.
108. Unno K, Furushima D, Hamamoto S, Iguchi K, Yamada H, Morita A, Pervin M, Nakamura Y. Stress-reducing effect of cookies containing matcha green tea: Essential ratio among theanine, arginine, caffeine and epigallocatechin gallate. *Heliyon.* 2019;5:e01653.
DOI:101016/j.heliyon.2019.e01653.
109. Morita A, Ikka T, Kunihiro A. Chemical composition of new shoots in the first crop season of “White Leaf Tea” cultivated in Japan. *Tea Res. J.* 2011;111:63–72.
110. Unno K, Furushima D, Nomura Y, Yamada H, Iguchi K, Taguchi K, Suzuki T, Ozeki M, Nakamura Y. Antidepressant Effect of Shaded White Leaf Tea Containing High Levels of Caffeine and Amino Acids. *Molecules.* 2020;25:3550,
DOI:10.3390/molecules25153550.
111. Rothenberg DO, Zhang L. Mechanisms Underlying the anti-depressive effects of regular tea consumption. *Nutrients.* 2019;11:1361.
112. John J, Kodama T, Siegel JM. Caffeine promotes glutamate and histamine release in the posterior hypothalamus. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2014;307:704–710.
113. Chou CW, Huang WJ, Tien LT, Wang SJ. (-)-Epigallocatechin gallate, the most active polyphenolic catechin in green tea, presynaptically facilitates Ca²⁺-dependent glutamate release via activation of protein kinase C in rat cerebral cortex. *Synapse.* 2007;61:889–902.