Potential Role of Neuroactive Tryptophan Metabolites in Central Fatigue: Establishment of the Fatigue Circuit

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ABSTRACT: Central fatigue leads to reduced ability to perform mental tasks, disrupted social life, and impaired brain functions from childhood to old age. Regarding the neurochemical mechanism, neuroactive tryptophan metabolites are thought to play key roles in central fatigue. Previous studies have supported the "tryptophan-serotonin enhancement hypothesis" in which tryptophan uptake into extensive brain regions enhances serotonin production in the rat model of exercise-induced fatigue. However, serotonin was transiently released after 30 minutes of treadmill running to exhaustion, but this did not reflect the duration of fatigue. In addition, as the vast majority of tryptophan is metabolized along the kynurenine pathway, possible involvement of the tryptophan-kynurenine pathway in the mechanism of central fatigue induction has been pointed out. More recently, our study demonstrated that uptake of tryptophan and kynurenine derived from the peripheral circulation into the brain enhances kynurenic acid production in rat brain in sleep deprivation–induced central fatigue, but without change in serotonin activity. In particular, dynamic change in glial-neuronal interactive processes within the hypothalamus-hippocampal circuit causes central fatigue. Furthermore, increased tryptophan-kynurenine pathway activity in this circuit causes reduced memory function. This indicates a major potential role for the endogenous tryptophan-kynurenine pathway in central fatigue, which supports the "tryptophan-kynurenine enhancement hypothesis." Here, we review research on the basic neuronal mechanism underlying central fatigue induced by neuroactive tryptophan metabolites. Notably, these basic findings could contribute to our understanding of latent mental problems associated with central fatigue.

KEYWORDS: Central fatigue, glial-neuronal interactions, neuroactive tryptophan metabolites

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Introduction

Are you tired? The precipitating factors of tiredness may involve brain overactivity at night, qualitative change in work habits, and brain exhaustion. The common factor predisposing one to fatigue is aging.[1] However, an epidemiological study reported that many students worldwide suffer from brain fatigue and sleep deprivation.[2] Under these conditions, concentration and intelligence are reduced, additionally leading to autonomic symptoms and the risk of depressive symptoms.[2] It is surprising that not only adults but also children suffer behavioral and brain function impairment due to fatigue. In a society coping with ongoing fatigue, it is important to identify the fatigue mechanism that can effectively mitigate fatigue-related cognitive decline and brain dysfunction.

Fatigue is mainly divided into central (brain or mental) fatigue, peripheral (muscle) fatigue, and infection fatigue and differs with respect to molecular causal factors. Several factors affecting each type of fatigue are depicted in Figure 1. Among these fatigue types, central fatigue and infection fatigue lead to complex exhaustion, and recovery is difficult without sufficient rest and supplements (medications) to block fatigue. In this review, we focused specifically on central fatigue because it closely relates to everyday life and the experience of most of us. Central fatigue is implicated in chronic fatigue syndrome (CFS; myalgic encephalomyelitis) pathology[3] and leads to reduced mental task performance,[4,5] disrupted social life,[2,3] and impaired brain functions,[6,7] throughout life from childhood to old age. Here, an understanding of the fatigue mechanism is required to mitigate fatigue. Therefore, it is very interesting that a growing body of evidence shows that central fatigue can be explained by neurochemical mechanisms involving "tryptophan."

Tryptophan, which is the precursor of serotonin and kynurenine, is an essential amino acid that produces intense changes in mood[8] and fatigue.[9] Moreover, tryptophan is involved in the development of areas of the brain associated with behavioral functions.[10,11] In a rat model of tryptophan restriction, a significant decrease in 5-bromo-2-deoxyuridine-positive cells was seen in the subgranular zone of the dentate gyrus; in addition, *c-Fos*-positive nuclei density was decreased in the prefrontal cortex, hippocampus, and amygdala, most likely suggesting a decrease in neurogenesis in the dentate gyrus by tryptophan restriction.[11] Furthermore, rats with reduced tryptophan intake had higher running performance compared with rats with enhanced tryptophan intake and decreased level of extracellular tryptophan in the striatum.[12] Therefore, it is clear that tryptophan in the brain is involved in fatigue. Also, this evidence supports previous findings that tryptophan ingestion led to subjective drowsiness, fatigue, and dullness of sensation in humans.[13] In addition, an electrophysiological study reported that elevated tryptophan concentration suppresses neuron firing.[14] Furthermore, administration of tryptophan not only

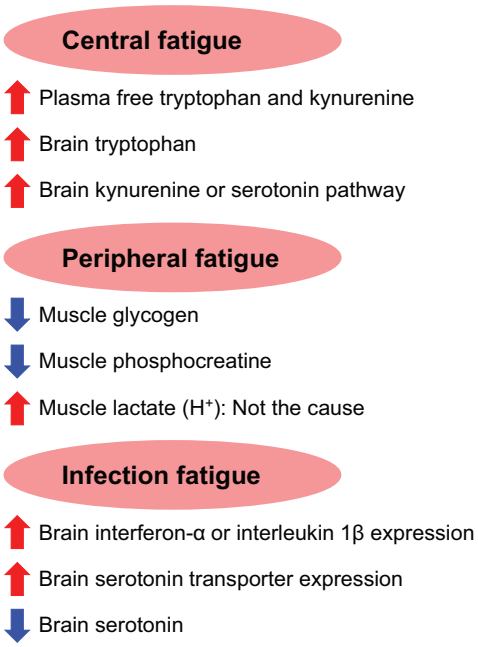

Figure 1. Factors affecting each type of fatigue.
Fatigue is mainly divided into 3 types: central fatigue, peripheral fatigue, and infection fatigue. Because of their neurochemical mechanisms, recovery from central fatigue and infection fatigue is more difficult without sufficient rest and supplements (or medicines) relative to peripheral fatigue.

caused fatigue but also decreased Morris water maze task performance during both memory acquisition and recall.[15] This evidence indicates that tryptophan could play a role in triggering central fatigue.

Previous studies have reported the "tryptophan-serotonin enhancement hypothesis" of central fatigue, which posits that central fatigue stems from increased passage of tryptophan into the brain and thus from higher levels of serotonin in the brain.[16-20] However, it is notable that outside of serotonin synthesis, the vast majority of tryptophan is metabolized via the kynurenine pathway into kynurenic acid and quinolinic acid[21] (Figure 2). Very recently, the "tryptophan-kynurenine enhancement hypothesis" has been proposed to explain the mechanism of central fatigue[15,22-25] and has been supported by evidence including reduced spontaneous motor activity in response to kynurenic acid administration, impaired memory performance in response to co-administration of kynurenic acid plus quinolinic acid,[15] and elevated concentrations of tryptophan and kynurenic acid in the brain under sleep deprivation–induced central fatigue conditions.[22,24]

The aim of this review is to summarize the neuroimaging, psychological, and neurochemical evidence for central fatigue triggered by neuroactive tryptophan metabolites. This work could contribute to our understanding of the latent mental problems associated with central fatigue.

Fatigue-Related Brain Changes

Fatigue is often observed in patients suffering from bone fracture,[9] cancer,[26] coronary heart disease,[27] stroke,[28] depression,[29] and neurodevelopmental disorders.[30] Thus, most of us experience fatigue and it may delay recovery from various pathological conditions.

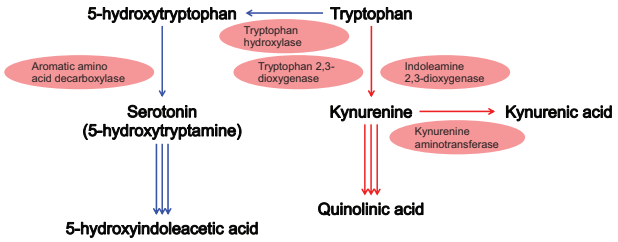

Figure 2. The neuroactive tryptophan pathway and metabolites.
In mammals, as only about 5% of tryptophan is catabolized via the serotonin pathway, the vast majority of tryptophan is metabolized in the kynurenine pathway, which is the precursor pathway for the synthesis of the neuroinhibitory molecule, kynurenic acid, and neurotoxic molecule, quinolinic acid. Is the rate of the kynurenine pathway of tryptophan metabolism involved in central fatigue? If the tryptophan-kynurenine pathway is enhanced during central fatigue, does it lead to a reduction in cognitive functions and severe fatigue?

First, this section presents the types of fatigue and their neurochemical mechanisms. The types of fatigue mainly include central fatigue, peripheral fatigue, and infection fatigue. For example, most of us have often heard this dogma of exercise, because it has been pointed out that accumulation of lactic acid in muscles is associated with the induction of muscle fatigue.[31] However, lactic acid is converted to pyruvic acid in the presence of oxygen, and then it is used for the synthesis of adenosine 5′-triphosphate (ATP) after being metabolized by the Krebs cycle (tricarboxylic acid cycle). Thus, accumulation of lactic acid and intracellular acidosis have protective effects on the performance of fatigued muscles.[32] Moreover, lactic acid is fuel for the Krebs cycle.[33] This evidence suggests that lactic acid is not involved in triggering fatigue.

Second, central fatigue is implicated in the pathological condition known as CFS,[3] and cross-sectional studies of patients with CFS provide an accumulating body of evidence on brain function. Cook et al[5] reported that compared with healthy controls, CFS patients in middle age showed increased activation during paced auditory serial attention tasks in various cortical regions, for example, cerebellum, hippocampus, thalamus, superior temporal cortex, and inferior frontal cortex. In addition, Caseras et al[4] reported that compared with healthy controls, CFS patients showed increased activation of the medial frontal cortex during 1-back memory tasks and increased activation of the inferior temporal cortex and medial temporal cortex during 2- and 3-back verbal working memory tasks. Regarding the brain regions associated with working memory, accumulated evidence shows that activation of the frontal cortex, parietal cortex, thalamus, medial temporal regions, basal ganglia, and cerebellar regions is involved in the processing of working memory.[34-37] Therefore, it is thought that brain regions involved in processing working memory are more extensively activated in patients with CFS.[38] Consequently, CFS may be leading to overload in the brain, which is a complex process involving brain overactivity manifesting as a sense of exhaustion in life. In addition, a magnetic resonance imaging study in patients with CFS showed a reduction of white matter

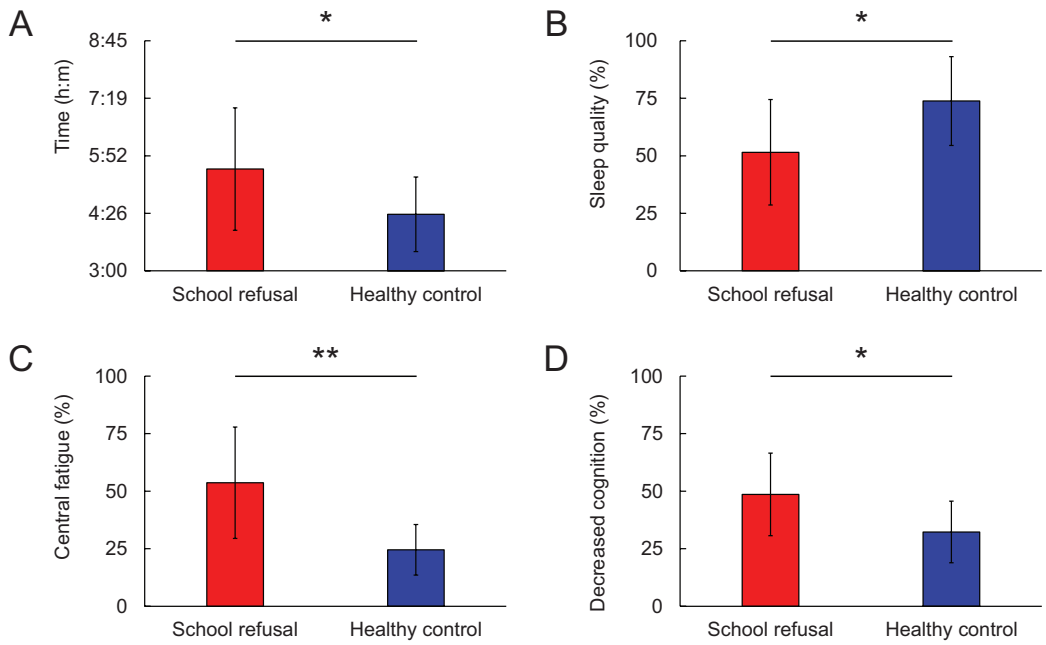

Figure 3. School refusal children showed higher levels of central fatigue and sleep derangement. (A and B): Compared with healthy control children, school refusal children showed a significant shift in the midpoint of sleep and lower sleep quality. (C and D): Moreover, induction of central fatigue and decreased cognition were significantly greater in school refusal children. The derangement of sleep and induction of central fatigue were higher for the school refusal children. *$P < .05$, **$P < .01$.

volume in the bilateral areas of the internal and external capsule and anterior midbrain, extending caudally into the bilateral pons, dorsally into the right prefrontal lobe, anteriorly into the inferior frontal lobe, and anteriorly into the right temporal lobe.[6] Moreover, the volume of the dorsal right prefrontal cortex in patients with CFS was negatively correlated with fatigue status.[39] These findings suggest that CFS leads to increase in cerebral-subcortical activation, despite decrease in cerebral-subcortical brain volume. At present, our laboratory is investigating associations between neuroactive tryptophan metabolites and brain functions in schoolchildren affected by central fatigue. If schoolchildren exhibit central fatigue due to neuroactive tryptophan metabolites, then their brain structure and function may have been impaired at an early age.

Overall, structural imaging investigation has found that the volume of brain in working memory–related regions is decreased in patients with CFS. On the contrary, functional imaging investigation has demonstrated that activation in these regions increased the processing of working memory content. Do the brain regions where there is derangement (cerebral-subcortical regions) manifest as central fatigue in children? It is possible that neuroactive tryptophan metabolite–related central fatigue leads to derangement of brain functions in children.

Sleep Disturbance–Related Central Fatigue

The induction of central fatigue is closely linked to abnormal sleep conditions. Sleep is divided into non–rapid eye movement sleep (REM) sleep and REM sleep, which are stages repeated about every 90 minutes, and then these sleep stages play a role in the recovery of brain function from fatigue. Insufficient sleep can lead to exhaustion in the brain, and cognitive and work efficiency can be impaired by strong daytime sleepiness.

Many schoolchildren suffer from fatigability, unrefreshing sleep, and reduced mental concentration.[2] Very recently, our laboratory demonstrated that although children with school refusal behavior may sleep 8 hours, they have lower sleep quality and a later sleep midpoint relative to the midpoint in healthy children (Figure 3A and B). Moreover, children who are school refusers have increased central fatigue and decreased cognition (Figure 3C and D). This result indicates that the sleep phase in school refusers is shifted to the daytime, and potential insufficient sleep is aggravated by night owl tendencies. Also, our laboratory demonstrated that sleep deprivation–induced central fatigue in rats led to impulsivity, hyperactivity, impaired spatial cognitive memory accuracy,[24] and decreased running performance,[40] as their condition approached complete exhaustion. As sleep deprivation is related to reduction in hippocampal neurogenesis[41] and memory retention,[42] it is possible that sleep deprivation–induced central fatigue highly influences neurogenesis and memory capacity. Furthermore, our laboratory demonstrated that central fatigue was positively correlated with disturbance of sleep rhythm (Figure 4A), and central fatigue was negatively correlated with sleep efficiency in schoolchildren (Figure 4B). These observations indicate that central fatigue becomes more severe as sleep disturbance progresses in schoolchildren.

Regarding associations between tryptophan metabolites and sleep conditions, Pocivavsek et al[43] reported that increased kynurenic acid in the brain after kynurenine injection caused

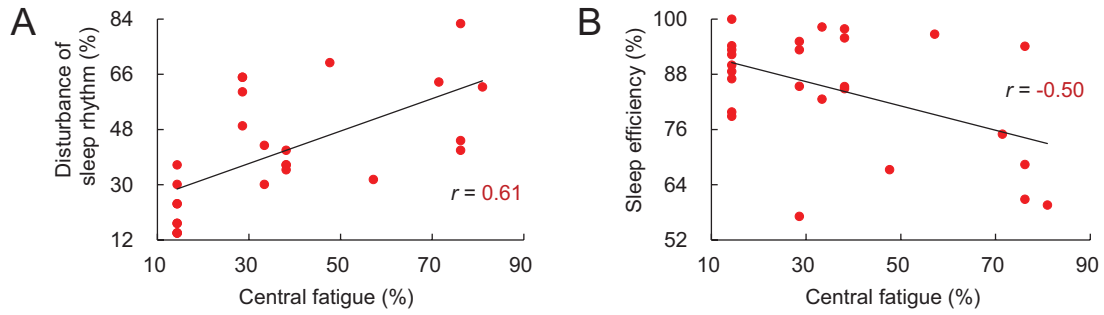

Figure 4. Central fatigue becomes more severe as sleep disturbance progresses. (A): In schoolchildren, central fatigue was positively correlated with disturbance of sleep rhythm ($r = 0.61$, $P = .001$). (B): In schoolchildren, central fatigue was negatively correlated with sleep efficiency ($r = -0.50$, $P = .01$). These results indicate that higher level of central fatigue is influenced by sleep disturbance.

total REM duration to decrease and total wake duration to increase. In addition, kynurenine injection impaired performance in the hippocampal-dependent contextual memory task. This finding suggests that kynurenic acid plays a key role in the molecular mechanism of sleep regulation. More notably, this finding provides a reason for investigating the role of neuroplasticity in central fatigue.

Taken together, previous studies suggest firm associations between central fatigue and sleep and lead to the conclusion that impaired cognition by sleep deprivation induces central fatigue. Thus, sleep deprivation–induced central fatigue may be associated with neuroplasticity reduction triggered by dynamic changes in neuroactive tryptophan metabolite levels.

Tryptophan-Related Central Fatigue

Tryptophan is an essential amino acid that binds to albumin in the circulation, and blood contains both free and bound tryptophan. Non-esterified fatty acids (NEFA) also compete for the same binding site. Increases in the levels of NEFA during exercise and postoperatively result in the dissociation of tryptophan from the tryptophan-albumin complex.[9,16] This leads to increased passage of free tryptophan into the brain through the blood-brain barrier (BBB) and thus to higher levels of serotonin in the brain through the enzymatic activity of tryptophan hydroxylase 2 (TPH2)[9,16] (see Figure 5). Moreover, branched-chain amino acids (BCAA) compete with free tryptophan for entry into the brain via the system L transporter L-type amino acid transporter 1 (LAT-1) located at the BBB.[44] If BCAA concentration decreases in the plasma, then the amount of free tryptophan entering the brain increases.

Yamamoto et al[9] reported that coronary artery bypass graft patients showed a higher than baseline concentration of plasma free tryptophan after surgery; the plasma free tryptophan to BCAA (free tryptophan/BCAA) ratio increased after surgery, whereas plasma albumin decreased; this suggests that elevated tryptophan levels are intimately associated with the induction of central fatigue in the brain. This evidence further supports previous findings.[45] Moreover, it has been speculated that Nagase analbuminemic rats could serve as an animal model of central fatigue because of their high plasma levels of free tryptophan as well as high free tryptophan/BCAA ratio in plasma.[9] In addition, Melancon et al[46] reported that older adults had an increased free tryptophan/BCAA ratio during sustained exercise compared with baseline. These findings provide evidence that tryptophan availability to the brain is elevated during fatigue and support for the hypothesis that serotonin synthesis is increased in central fatigue.

Similarly, tryptophan ingestion led to elevated free tryptophan in the blood[47,48]; lower activation in the postcentral gyrus, angular gyrus, inferior frontal gyrus, and inferior frontal sulcus during the Stroop task[48]; and induction of drowsiness and fatigue.[13] Furthermore, an increased passage of free tryptophan in the brain was shown to enhance serotonin synthesis during the acute-fatigue stage[49] and elevate serotonin transporter expression in the hippocampus and prefrontal cortex, and reduced serotonin$_{1A}$ receptor expression was shown to increase serotonin concentration in the brain during the chronic fatigue stage.[50] In addition, Yamamoto et al[15] reported that in tryptophan-treated rats, the acquisition of memory was delayed by tryptophan in the initial learning stage during the Morris water maze task. In the relearning phase of the Morris water maze task followed by memory evaluation using the probe test, tryptophan prolonged mean goal latency, suggesting that it qualitatively decreased learning recall. Thus, administration of tryptophan not only caused fatigue but also decreased cognition, including memory acquisition. However, little is known about the role of tryptophan receptors in central fatigue.

Taken together, the facts indicate that an increase in the plasma concentration of free tryptophan can result in exercise-induced fatigue and postoperative-induced fatigue. This leads to increased passage of tryptophan into the brain through the BBB and thus to higher levels of serotonin in the brain. Therefore, central fatigue can be controlled by the excessive levels of tryptophan at the BBB.

Tryptophan-Serotonin Enhancement Hypothesis

In 1987, Newsholme et al[51] hypothesized that fatigue was caused by an increase in the plasma level of tryptophan and thereby in the brain level of neurotransmitter serotonin, which had negative effects on arousal, lethargy, sleepiness, and mood. Outside of fatigue, serotonin has a role in the

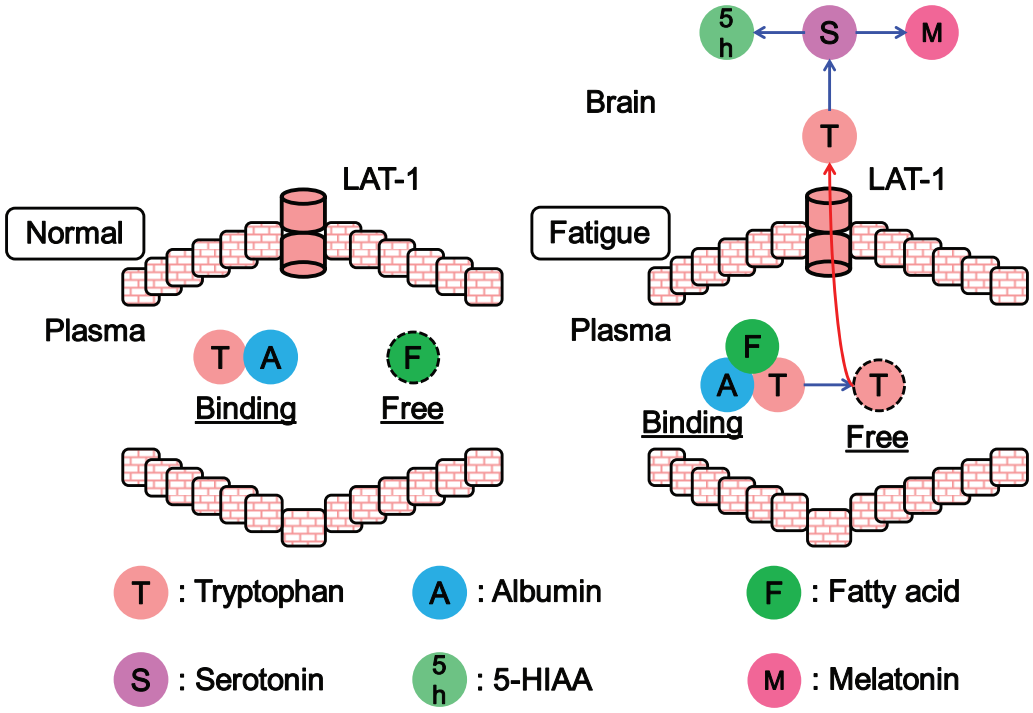

Figure 5. The uptake of peripheral tryptophan by the brain.
Tryptophan binds to albumin in the blood under normal conditions. Non-esterified fatty acids (NEFA) also compete for the same binding site. An increase in the levels of NEFA results in the dissociation of albumin and tryptophan during exercise-induced fatigue and postoperative-induced fatigue. Then, free tryptophan is rapidly taken into the brain via system L transporter located on the surface of the blood-brain barrier, and thus leading to enhanced serotonin synthesis in the brain by the enzymatic activity of tryptophan hydroxylase 2. 5-HIAA indicates 5-hydroxyindoleacetic acid; LAT-1, L-type amino acid transporter 1.

pathophysiological mechanism of depression[52-54] and in brain development and neuroplastic regulation.[10,11,55] Although the neurochemical mechanism of central fatigue is often confused with that of depression and both depend on the influences of neuroactive tryptophan metabolites, these mechanisms are completely different. For example, Maurer-Spurej et al[56] reported that depressive patients showed lower level of serotonin in platelets. Also, Ogawa et al[57] provided evidence of lower blood levels of tryptophan in depressive patients. Thus, tryptophan depletion may induce depressive symptoms,[58-60] suggesting that tryptophan depletion and thereby lower serotonin synthesis decrease neuroplasticity.[10,11,55] Moreover, depressive patients showed a decreased kynurenic acid level in the plasma compared with healthy controls.[61] As higher levels of neuroactive tryptophan metabolites have a known relationship to the induction of central fatigue, the basic neurochemical mechanism in central fatigue differs from that in depression. Fatigue is the earliest manifestation of impaired health outcomes and quality of life, and prolonged fatigue may be linked to induction of depressive symptoms in the future.[2,62,63]

In addition, there is an accumulating body of evidence showing serotonin activation is associated with central fatigue. Blomstrand et al[64] investigated the different concentrations of serotonin and its metabolite 5-hydroxyindoleacetic acid over a wide swath of the brain in resting and treadmill-exercised rats. The rats in the treadmill-exercised group, when compared with rats in the resting group, showed higher concentrations of serotonin and 5-hydroxyindoleacetic acid in the brain stem and hypothalamus and higher concentration of 5-hydroxyindoleacetic acid but not serotonin in the hippocampus and striatum, but both groups showed similar concentrations of these compounds in the cortex and cerebellum. This result indicates that sustained exercise generates central fatigue that leads to an increase in serotonin concentration, specifically in the hypothalamus and brain stem. This evidence has been supported by previous findings that led to the tryptophan-serotonin enhancement hypothesis.[16-20] In contrast, Yamamoto et al[15] used *in vivo* microdialysis to show that serotonin was transiently released after 30 minutes of treadmill running to exhaustion, but this did not reflect the duration of fatigue. Moreover, after supplementation with 2 μM L-tryptophan, the serotonin released in response to 30 mM K^+ was immediately taken up by nerve terminals at 60 minutes and the gradually reabsorbed serotonin was rapidly metabolized to 5-hydroxyindoleacetic acid, which returned the concentration of serotonin to its original level at 90 minutes. These findings show that released serotonin is quickly reabsorbed and subsequently metabolized to 5-hydroxyindoleacetic acid, suggesting that serotonin has no effect on fatigue. As Nagase analbuminemic rats are known to have lower blood levels of albumin,[65] they had higher extracellular tryptophan concentrations and lower extracellular 5-hydroxyindoleacetic acid concentrations when fatigued by treadmill running.[15] To test whether the neuromodulatory functions of the tryptophan-serotonin pathway are activated by a tryptophan receptor agonist, our laboratory intraperitoneally injected the tryptophan receptor agonist D,L-β-(1-naphthyl)alanine. The rats injected with D,L-β-(1-naphthyl) alanine had lower spontaneous locomotor activity in the open field than rats injected with saline (Figure 6A and B). In contrast, rats injected with D,L-β-(1-naphthyl)alanine had decreased serotonin

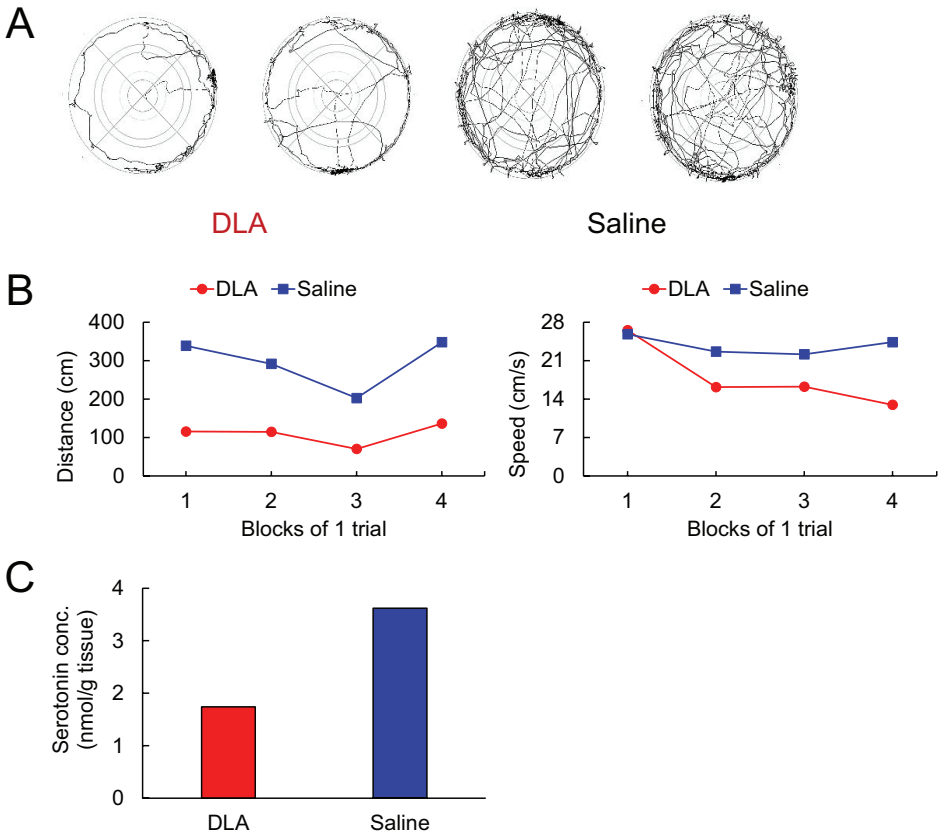

Figure 6. The effect of tryptophan receptor agonist administration on biobehavioral activities. (A and B): Rats administered DLA had lower spontaneous locomotor activities in the open field than rats administered saline. (C): Serotonin concentration in the hypothalamus decreased in rats injected with DLA compared with rats injected with saline. This result indicates that tryptophan receptor agonist induces the reduction of locomotor activity and serotonin level, although most studies have reported that serotonin activity in the brain is associated with central fatigue. It is possible that tryptophan-serotonin pathway is not involved in central fatigue. DLA indicates D,L-β-(1-naphthyl)alanine.

concentrations in the hypothalamus (Figure 6C). It is possible that the enhancement of the tryptophan-serotonin pathway in the brain is not involved in the rat's central fatigue.

Some human studies have failed to support the role of tryptophan-serotonin enhancement in central fatigue. Some studies have reported that administration of serotonin reuptake inhibitors reduces performance capability.[66-68] However, most studies have demonstrated that serotonin reuptake inhibitors do not negatively affect central fatigue.[69-73] For example, Meeusen et al. used a double-blind randomized crossover design to administer the serotonin reuptake inhibitor fluoxetine to athletes before exercise for 90 minutes. The results showed that exercise performance is not influenced by fluoxetine, although some plasma hormones indicated a central effect of the drug. This evidence supports that serotonin does not exacerbate exercise-induced central fatigue. However, most studies on central fatigue have been focused on the neurochemical mechanism of exercise-induced fatigue. In fact, central fatigue induced by chronic sleep disorders reportedly affects 40% to 80% of school children, causing school attendance difficulties, facilitating psychiatric disease development,[2,74] and contributing to brain dysfunction development.[75] To clarify the neurochemical mechanism of neuroactive tryptophan metabolites in central fatigue associated with sleep disorder, our laboratory established a rat model of central fatigue induced by chronic sleep disorder (CFSD).[40] In CFSD rats, free tryptophan was taken up into the brain via the BBB and subsequently led to an increase in presynaptic levels of tryptophan in the hypothalamus and hippocampus and a decrease in presynaptic levels of serotonin in the hypothalamus, hippocampus, cerebral cortex, striatum, and medulla oblongata.[24] In addition, psychomotor activity and social interaction were disrupted,[40] spatial cognitive memory accuracy impaired, and hyperactivity and impulsivity increased.[24] Therefore, it was concluded that CFSD rats could serve as an animal model of central fatigue associated with sleep disorder, given their high hippocampal-hypothalamic levels of tryptophan, high blood levels of free tryptophan, and inability to synthesize serotonin. Thus, our findings disprove the hypothesis that serotonin has a role in central fatigue.[15,22,24,40] However, little is known about temporal changes in neuroactive tryptophan metabolite levels in the brain of the CFSD rat.

Moreover, tryptophan in the blood at high concentration usually needs to enter the brain via LAT-1 at the BBB.[44] However, tryptophan may be able to enter the brain without LAT-1. For example, there is some evidence that psychological

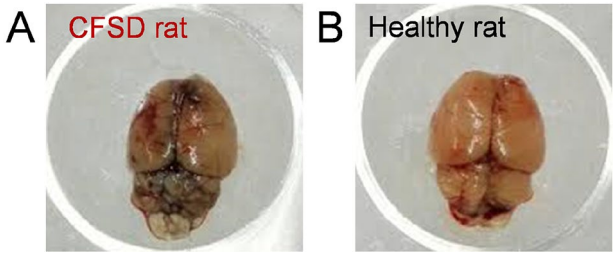

Figure 7. Disruption of the blood-brain barrier (BBB) by sleep deprivation–induced central fatigue. The breakdown of the BBB can be estimated by quantification of extravasated Evans Blue in the brain. Using this method, extravasated Evans Blue content in the whole brain increased in central fatigue induced by (A) chronic sleep disorder (CFSD) rats compared with (B) healthy rats. This result indicates that central fatigue could lead to increased BBB permeability, suggesting barrier breakdown. Perhaps, tryptophan and other substances can freely enter the brain without the assistance of some transporter.

stress increases the permeability of the BBB, suggesting that some compounds can enter the brain without transporters.[76,77] The breakdown of the BBB can be assessed by quantification of extravasated Evans Blue in the brain.[78,79] With this method, our laboratory investigated the permeability of the BBB in CFSD rats. The results showed that brain tissue from CFSD rats, unlike from healthy rats, turned blue in color as a result of Evans Blue leakage (Figure 7). This finding indicates that central fatigue can lead to breakdown of the BBB, suggesting that the excessive amount of plasma tryptophan can facilitate its entry into the brain without LAT-1 mediation or may cause the breakdown of LAT-1 functions under severe fatigue conditions, and lead to accelerated metabolism of tryptophan in the brain to kynurenine and serotonin.

However, evidence that lower levels of serotonin are associated with the precipitating factors of fatigue, especially CFS, is accumulating. The clinical symptoms of CFS are characterized by autonomic, neuroendocrine, and immune function impairment.[80,81] Previous studies have reported that in infection-related CFS, inflammatory cytokines in the brain can lead to reduced behavioral performance, disrupted hypothalamic-pituitary-adrenal axis, and impaired peripheral cellular immunity.[82,83] Therefore, it is speculated that increased cytokine levels and decreased serotonin level can be involved in pathogen-induced CFS.

The polyriboinosinic-polyribocytidylic acid (poly-I:C), a virus-mimicking synthetic double-stranded RNA, is very useful for understanding immunologically induced fatigue. In a rat model of poly-I:C-induced fatigue, spontaneous wheel running decreased until day 8 after poly-I:C injection.[83,84] In addition, there was increased expression of interleukin 1β messenger RNA (mRNA) in the cerebellum, medial preoptic area, lateral preoptic area, paraventricular hypothalamic nucleus, and lateral hypothalamic, as well as increased expression of interferon-α mRNA.[83,84] As patients with CFS showed impaired cytokine production and immune functions such as an increased level of interferon-α in the cerebrospinal fluid[85] and decreased natural killer cell activity,[86] expression of interferon-α in the brain may be associated with poly-I:C-induced fatigue as well as CFS. Moreover, interferon-α has been shown to upregulate the transcription of serotonin transporter mRNA in cultured cell lines.[87] Katafuchi et al[84] reported that the expression of serotonin transporter in the brain was enhanced by interferon-α in poly-I:C-induced fatigue rats and decreased the extracellular levels of serotonin in the medial prefrontal cortex. These findings suggest that serotonin transporter overexpressed in the medial prefrontal cortex scavenges serotonin, subsequently leading to the reduction in serotonin levels by poly-I:C injection. This is theorized to cause infection-related fatigue, which is the "serotonin reduction hypothesis."

Furthermore, there is accumulating evidence for the involvement of glial cell cytokines in inflammatory fatigue. For example, interleukin 1β, a proinflammatory cytokine involved in poly-I:C-induced fatigue, is produced by activated microglia. Moreover, Ifuku et al[83] demonstrated that direct application of poly-I:C to primary cultured microglia enhances their expression of interleukin 1β. This finding suggests that the injection of poly-I:C leads to increased expression of interleukin 1β in microglia from the rat fatigue model. This evidence supports the finding that microglial activation inhibition by minocycline suppresses increased interleukin 1β expression and CFS induction. As activation of microglia plays an important role in neuroimmunological diseases, it is possible that microglial activation is involved in inflammatory fatigue. Furthermore, microglia closely interact with astrocytes in the brain.[88,89] Although interferon-α has no effect on serotonin transporter expression in astrocytes, interleukin 1β increases expression of serotonin transporter in primary cultured astrocytes.[83] It has been shown that injection of poly-I: C induces expression of serotonin transporter in astrocytes, but not in microglia. Thus, synthesis of interleukin 1β by microglial activation leads to enhanced expression of serotonin transporter in astrocytes during poly-I:C-induced fatigue, subsequently reducing serotonin levels in the brain. Importantly, we need to separate not only neurons but also glia in future research to clarify the neurochemical mechanism in central fatigue.

Taken together, these findings suggest that in the rat model of treadmill exercise–induced fatigue, levels of tryptophan and serotonin are increased in the brain stem and hippocampus. However, central fatigue in most people is closely correlated in everyday life. For example, many schoolchildren experience central fatigue induced by chronic sleep disorders, which causes them to have school attendance difficulties. To resolve the neurochemical mechanism of sleep disorder–induced central fatigue, our laboratory established a new rat model of central fatigue induced by chronic sleep disorder, that is, CFSD. The rat model of CFSD can be characterized by increasing tryptophan uptake into the brain and lack of serotonin synthesis. In addition, once transported into the brain, tryptophan is readily metabolized to kynurenine. This evidence points to a key role for the tryptophan-kynurenine pathway in the behavioral and biochemical mechanism of central fatigue.

Tryptophan-Kynurenine Enhancement Hypothesis

Previous studies have supported the tryptophan-serotonin enhancement hypothesis, in which tryptophan uptake into the brain enhances serotonin production, as demonstrated in the rat model of sustained-exercise fatigue.[16-20] Studies in humans also support the hypothesis by showing higher plasma levels of free tryptophan during postoperative-induced fatigue[9,45] and exercise-induced fatigue.[20] However, 5% and 95% of tryptophan, when taken up into the brain, are metabolized along 2 pathways, the serotonin pathway and the kynurenine pathway, respectively.[90] Kynurenine is metabolized to quinolinic acid and kynurenic acid. Quinolinic acid is neurotoxic in the central nervous system[91] and has been shown to be present at higher level in patients with CFS.[92] Quinolinic acid is an *N*-methyl-D-aspartic acid (NMDA) receptor agonist and causes excitotoxic neuronal death.[93] On the contrary, kynurenic acid is an endogenous astrocyte-derived neuromodulator[21] and is implicated in the cognitive process and pathophysiological mechanism in some diseases.[24,94-96] Mackay et al[97] revealed that after tryptophan loading, brain-damaged patients had higher levels of kynurenic acid than healthy controls. Also, kynurenic acid has been reported to act as an antagonist of both NMDA and α7-nicotinic acetylcholine (α7nACh) receptors.[21,98] Therefore, it is considered to take part in glutamatergic and cholinergic neurotransmission in the central nervous system.[99,100] These findings suggest that brain dysfunction in central fatigue may be associated with tryptophan-kynurenine pathway upregulation.

There is an accumulating body of evidence of the kynurenine pathway's association with central fatigue. Yamamoto et al[15] reported that the microinjection of 3 nmol of kynurenic acid into the third cerebral ventricle decreased physical and open-field and rearing activity, and injection of kynurenic acid at 0.25 mM caused a dose-dependent increase in fatigue induced by running. In addition, quinolinic acid alone or coadministered with kynurenic acid produced a decrease in memory recall and retention in the Morris water maze task. These findings suggest that not only tryptophan but also its metabolites kynurenine and quinolinic acid decrease spatial memory performance and lead to the kynurenic acid-quinolinic acid hypothesis that central fatigue arises due to a rapid increase in concentrations of active neurometabolites. In addition, our laboratory performed the experiment in rats intraperitoneally injected with kynurenine (100 mg/kg).[24] The rats showed increased kynurenic acid synthesis in the hippocampus compared with rats injected with saline and suppressed recall of retained spatial cognitive memory, but not the acquisition of memory. This evidence proves that peripherally administered kynurenine enters the brain via the BBB and thereby increases kynurenic acid synthesis in the hippocampus. Moreover, kynurenic acid is associated with reduction in glutamate levels.[21,99,100] Reduction in glutamate levels has been implicated in spatial cognitive memory loss[101] and impaired social behavior.[102] Therefore, reduction in glutamate levels by increasing kynurenic acid levels may be causing the inaccurate recall of retained memory in central fatigue. However, little is known about the neuromodulatory functions of glutamate in the brain during central fatigue. Furthermore, elevated kynurenine concentration suppresses dopamine release into the synaptic cleft.[103] Our laboratory demonstrated the reduction in concentration of dopamine and its metabolite 3,4-dihydroxy-phenylacetic acid in presynaptic neurons of the hypothalamus and hippocampus in CFSD rats compared with healthy rats, suggesting the possibility that elevated concentration of kynurenic acid in presynaptic neurons may suppress dopamine release from the presynaptic neurons.[24] Overall, central fatigue may be caused by metabolism of kynurenine to kynurenic acid. Nevertheless, the link between levels of endogenous kynurenine metabolites in the peripheral and central nervous system and central fatigue is not fully understood, nor have associations between the central fatigue mechanism and kynurenine metabolites been firmly established.

Regarding kynurenine *in vivo*, 40% of kynurenine in the brain is produced in the central nervous system, whereas 60% has its origin in the peripheral nervous system.[96,104] Also, brain entry of peripheral kynurenine is facilitated via neutral amino acid transporters expressed in the BBB.[96,104] Moreover, kynurenine is metabolized into kynurenic acid or quinolinic acid, with the first step (metabolism into kynurenic acid) involving catalytic reaction with kynurenine aminotransferases (KATs) localized in astrocytes.[105] Notably, the endogenous kynurenine metabolic pathway in the peripheral and central nervous systems changes during fatigue. Recently, Strasser et al[106] reported that exhaustive aerobic exercise in young adults reduced tryptophan concentration and increased kynurenine levels in blood while increasing the kynurenine/tryptophan ratio. This result suggests that the enhancement of kynurenine may be partly associated with exercise-induced fatigue, but the study did not examine the association with fatigue score, although low tryptophan levels followed by intense exercise may diminish its supply to the brain and thereby limit its availability for serotonin production. Regarding this counterargument, it is possible that plasma levels of kynurenine are rapidly increased by catalysis of free tryptophan during exercise, but without serotonin synthesis in the brain. Also, the association between fatigue and elevated blood levels of kynurenine supports recent findings in chronic hemodialysis patients.[107]

Moreover, strong evidence was obtained for the role of the endogenous tryptophan-kynurenine pathway in the rat model of central fatigue. Very recently, our laboratory reported that CFSD rats showed higher blood levels of tryptophan and kynurenine compared with healthy rats.[24] This suggests that tryptophan and kynurenine, which are present as free forms at increased levels in the blood, enter the brain during central fatigue via the BBB in a synergestic manner (Figure 8). In addition, our laboratory first demonstrated that tryptophan concentrations in presynaptic neurons of the hypothalamus and hippocampus and kynurenine-to-kynurenic acid metabolism were drastically increased in

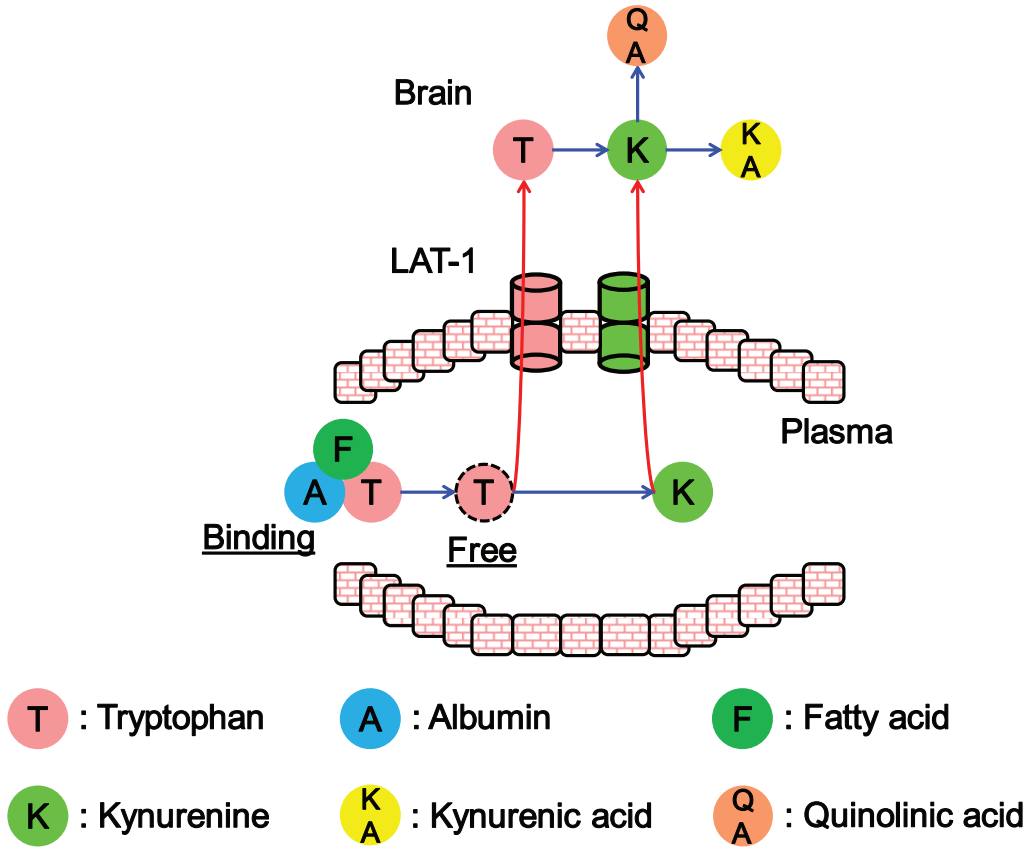

Figure 8. Metabolism of tryptophan to kynurenine during central fatigue.
Tryptophan is catabolized along the kynurenine pathway by tryptophan dioxygenase (TDO) or indoleamine dioxygenase in the liver, and then crosses the blood-brain barrier (BBB) to be rapidly taken up in the brain. Also, tryptophan may be directly metabolized to kynurenine by the enzymatic activity of TDO in the brain. In central fatigue, tryptophan and kynurenine enter the brain via the BBB in a synergetic manner. LAT-1 indicates L-type amino acid transporter 1.

CFSD rats, but this was not true for serotonin metabolism. These findings provide convincing supportive evidence of the tryptophan-kynurenine enhancement hypothesis in central fatigue and demonstrate the presence of peripheral presynaptic tryptophan and kynurenine in the hypothalamus-hippocampal circuit and the production of the tryptophan-kynurenine-kynurenic acid amplification effect during central fatigue. Thus, our laboratory provided the first evidence of the involvement of a tryptophan-kynurenine pathway mechanism at neuronal junctions in the transitional zone of the peripheral and central nervous systems during central fatigue. However, little is known about the central effect of quinolinic acid on central fatigue.

Regarding the activation of key enzymes in tryptophan metabolism, peripheral kynurenine is produced by the catalytic reaction of free tryptophan with indoleamine-2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) in the liver.[108,109] Then, it crosses the BBB to be rapidly taken up in the brain.[110] However, a previous study found that brain tissue did not express IDO and TDO.[109] In contrast, 1 study in TDO$^{-/-}$ mice reported the critical role of TDO in hippocampal neurogenesis, hippocampal neuron maintenance, and anxiety-related behavior.[111] In addition, the immature dentate gyrus in adult alpha-CaMKII hetero-knockout mice showed decreased expression of TDO mRNA, suggesting that decreased expression of TDO was associated with the impairment of working memory and mood regulation.[112] Furthermore, Kanai et al[113] found that TDO was expressed in the hippocampus and cerebellum, suggesting that TDO is locally expressed and regulated in the brain and therefore may be associated with hippocampal and cerebellar development and function. Tryptophan and its neuroactive metabolite kynurenine, which is converted by TDO or IDO, are involved in increased production of nerve growth factor in astrocytes and promote hippocampal and cerebellar development.[10,11,114-117] Furthermore, besides catalyzing tryptophan with IDO, TDO plays a pivotal role in the homeostasis of tryptophan metabolites in the peripheral and central nervous systems.[105,113,118] Thus, the impairment of TDO expression in the brain and liver may be associated with neuropathological disorder. However, little is known about the associations between the key enzymes of tryptophan metabolism and central fatigue.

Taken together, our findings in CFSD rats show that tryptophan and kynurenine, which are present as free forms and increased in blood, enter the brain via the BBB in a synergetic manner, where their presence in the presynaptic neurons of limited brain regions amplifies the effect of the tryptophan-kynurenine-kynurenic acid pathway during central fatigue. Our first evidence is supported by recent reports[119] that propose a role for not only serotonin but also kynurenine. Furthermore, it is necessary to clarify the role of glial neuroactive tryptophan metabolites in central fatigue.

The Establishment of the Fatigue Circuit

Recent studies have supported the tryptophan-kynurenine enhancement hypothesis of central fatigue.[15,22-25] For example, our laboratory findings demonstrated the involvement of the amplification effect of tryptophan-kynurenine-kynurenic acid pathway factors in central fatigue.[24] However, the brain contains 10% neurons and 90% glial cells,[120] and thus the targets in previous analyses of central fatigue–associated neuroactive tryptophan metabolites are both glial cells and neurons. Kynurenic acid synthesis has long been thought to take place in astrocytes, and few studies have focused on other glial cells. Although oligodendrocytes protect neuronal axons by forming a myelin sheath around the axons to allow saltatory conduction of nerve action potentials,[121] previous studies have reported that oligodendrocytes may also take part in neurotransmission and synaptic activity.[122,123] Wejksza et al[124] demonstrated that KAT enzymes are present in an oligodendrocyte cell line and produce oligodendrocytic kynurenic acid from L-kynurenine in a concentration- and time-dependent manner. Kynurenic acid synthesis in an oligodendrocyte cell line has been shown to be decreased in a concentration-dependent manner by L-tryptophan. These findings suggest that oligodendrocytes may be associated with the regulation of kynurenic acid balance in the brain. Moreover, the number of O4-positive cells in oligodendrocyte culture reduced after incubation with quinolinic acid.[125] This evidence suggests that elevated quinolinic acid during neuropathological diseases may induce oligodendrocyte death. However, the results of previous studies on central fatigue do not provide clear evidence that induction in central fatigue is attributable to the characteristics of glial-neuronal interactive process. Therefore, how tryptophan and kynurenine, present in the periphery, behave as inducers of central fatigue in the glial-neuronal circuit remains unknown.

Our laboratory identified a tendency for elevation in tryptophan concentration in oligodendrocytes during central fatigue.[24] Although it is difficult to interpret the meaning of the elevated tryptophan concentration and presence of kynurenic acid in oligodendrocytes within the hypothalamus-hippocampal circuit during central fatigue, with much remaining unknown as to the finding, the association of oligodendrocytic kynurenic acid and elevated oligodendrocytic tryptophan with the pathogenesis of central fatigue is still possible. For example, myelin sheath damage by impairment of the oligodendrocytic system induces cognitive dysfunction via lowered nerve conduction velocity.[126,127] Furthermore, an electrophysiological study reported that elevated tryptophan concentration suppresses neuron firing.[14] In central fatigue, an increase in tryptophan concentration in oligodendrocytes may inhibit saltatory conduction of nerve action potentials in hypothalamic and hippocampal neurons, forming a basis for axonal disorder and cognitive dysfunction. In detail, dynamic change in glial-neuronal interactive processes within the hypothalamus-hippocampal circuit causes central fatigue, and increased tryptophan-kynurenine pathway activity in this circuit causes reduced cognitive function. However, little is known about the functional role of astrocytes and microglia in central fatigue.

Taken together, our study suggests that uptake of peripheral kynurenine and tryptophan into the brain enhances kynurenic acid production in the brain, and the combination of the 3 factors has a synergetic effect on the neuronal junctions between peripheral and central nervous system and its role in central fatigue, triggering cognitive dysfunction. Thus, our laboratory has provided the first evidence that the fatigue circuit is responsive to tryptophan-kynurenine-kynurenic acid pathway signals generated at neuronal-neuronal and glial-neuronal synapses linking the peripheral and central nervous systems (Figure 9).

Tryptophan Metabolites and Fatigue Associated With Neurodevelopmental Disorders

Recently, our laboratory focused on the association between tryptophan metabolites and neurodevelopmental disorders because of recent studies reporting that patients with neurodevelopmental disorders often have a history of fatigue, daytime tiredness, and daytime sleepiness.[30,128-130] Our previous study demonstrated that autistic symptoms were correlated with higher levels of 3-methoxy-4-hydroxyphenylglycol and lower levels of 5-hydroxyindoleacetic acid,[131] suggesting that dynamic changes in the levels of tryptophan metabolites may be associated with symptoms of neurodevelopmental disorders, including sleep disturbances and fatigue.

The recent evidence on central fatigue supports the role of the tryptophan-kynurenine pathway,[15,22-25,119] in which enhanced kynurenic acid production triggered by tryptophan and kynurenine uptake from the plasma into the brain, as demonstrated in CFSD rats, may then decrease the concentration of BCAA in the plasma.[24] Like patients with autism spectrum disorder (ASD), patients with homozygous branched-chain ketoacid dehydrogenase kinase (BCKDK) mutations had lower levels of BCKDK mRNA and protein, E1α phosphorylation, and plasma BCAA.[132] Thus, it is notable that tryptophan metabolic mechanisms are associated with fatigue symptoms in neurodevelopmental disorders. Moreover, Hakamada and Yamamoto reported that a rat model of neurodevelopmental disorder, Nagase analbuminemic rats, showed lower levels of serotonin in the prefrontal cortex, chronic enhancement of free tryptophan, chronic lack of BCAA in the plasma, and higher levels of inattention and hyperactivity/impulsivity.[133] Autistic traits were present in patients carrying deleterious homozygous mutations in the gene encoding solute carrier transporter 7a5, which is a large neutral amino acid transporter located at the BBB.[134,135] Furthermore, a previous study found that mice lacking TPH2 are defective in serotonin synthesis in the brain and display behavioral symptoms in ASD.[136] These findings suggest that imbalance between tryptophan and BCAA levels in the plasma by mutation of TPH2 and BCKDK gene may be associated with inefficient synthesis of

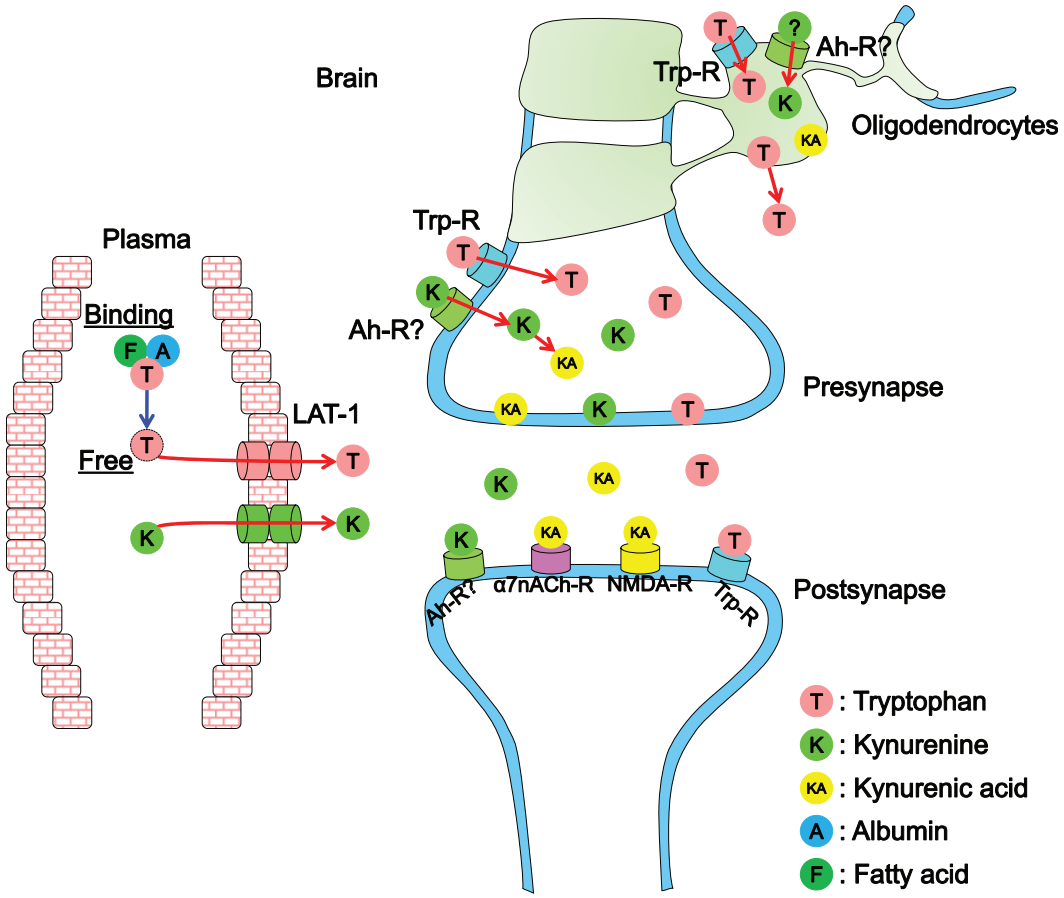

Figure 9. The role of the fatigue circuit: from blood to brain.
There are 3 stages of central fatigue induction. Stage 1 is marked by synergetic transfer of tryptophan and kynurenine from blood to brain. Stage 2 is indicated by the rise of brain tryptophan and kynurenine to excessive levels and detection of tryptophan-kynurenic acid pathway activity at the glial-neuronal interactive level within the hypothalamus-hippocampal circuit. Stage 3 is the impairment of cognitive functions. The fatigue circuit includes the tryptophan-kynurenine-kynurenic acid pathway signals generated at neuronal-neuronal and glial-neuronal synapses between the peripheral and central nervous systems. Enhancement of pathway activity triggers cognitive dysfunction. LAT-1 indicates L-type amino acid transporter 1.

serotonin in the brain and cause the fatigue symptoms in neurodevelopmental disorders.

Alleviating the Effect of Fatigue Using Supplements

The generation of neuroactive tryptophan metabolites at junctions of the peripheral and central nervous system leads to complex exhaustion, and recovery is difficult without sufficient rest and supplements to block central fatigue. In this section mainly, we introduce 2 candidate supplements and substances associated with the inhibition of neuroactive tryptophan metabolites.

BCAA supplements, including valine, leucine, and isoleucine, have been proposed to alleviate exercise-induced fatigue[15,44] and inhibit muscle atrophy.[137] The administration of BCAA improved running performance by decreasing extracellular tryptophan,[15] suggesting that BCAA inhibit intracerebral tryptophan release and uptake from the circulation. Moreover, rats on BCAA-supplemented diets showed a decreased kynurenic acid level in the brain.[94] These findings suggest that BCAA would be expected to exert an alleviating effect on fatigue by inhibiting metabolic activation of the tryptophan-kynurenine metabolic pathway. However, from the obesity perspective, supplementation of high-energy diets with BCAA may be associated with neurobehavioral impairment and obesity.[94]

Regarding other candidate substances, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) may be effective in fatigue reduction. Because BCH is a specific inhibitor of LAT activity, it may prevent tryptophan and kynurenine uptake from the circulation and thereby relieve fatigue. In addition, BCH activates glutamate dehydrogenase,[138,139] which is thought to play key roles in glutamate metabolism and the Krebs cycle. In CFSD rats, significant increases were seen in the brain level of kynurenic acid, and increase in kynurenic acid level may be associated with reduced glutamate level,[22,24] which plays a role in central fatigue by inhibiting NMDA and α7nACh receptors. Also, Choi et al[140] reported that treatment with BCH increased the levels of Krebs cycle intermediates, restored ATP levels, and enhanced the oxidation rate, suggesting that BCH treatment may promote the recovery of fatigued muscle. Furthermore, treatment with BCH increased run time in rats.[44] Therefore, we concluded that BCH may suppress the effect of increased tryptophan and kynurenic acid and decreased glutamate in the brain during central fatigue.

Regarding side effects, BCH has been shown to be nonlethal, without effect on body weight, weights of major organs, food intake, or physical appearance, and not to cause liver toxicity as determined by aspartate aminotransferase and alanine aminotransferase activity assays.[141] This evidence indicates that the pharmacological action of BCH may safely be taken to help prevent central fatigue.

Taken together, BCAA and BCH supplements may be used to suppress the enhancement of tryptophan-kynurenine metabolic pathway in the brain resulting from synergetic uptake of blood tryptophan and kynurenine. On the contrary, BCAA has side effects such as obesity and neurobehavioral impairment. Coadministration of BCAA and BCH to alleviate central fatigue remains to be explored.

Conclusions and Future Perspectives

Neuroactive tryptophan metabolites are a major cause of central fatigue. Previous studies have supported the tryptophan-serotonin enhancement hypothesis, in which tryptophan uptake into the brain enhances serotonin production in exercise-induced fatigue. However, the release of serotonin was transient after 30 minutes of treadmill running to exhaustion and did not reflect the duration of fatigue. In addition, as 95% of tryptophan metabolism is converted by the kynurenine pathway, possible involvement of the tryptophan-kynurenine pathway in central fatigue induction has been pointed out. More recently, our study demonstrated that uptake of tryptophan and kynurenine from the peripheral circulation into the brain enhances kynurenic acid synthesis in the brain in sleep deprivation–induced central fatigue, but without change in serotonin activity. In particular, dynamic change in glial-neuronal interactive processes within the hypothalamus-hippocampal circuit causes central fatigue, and increased tryptophan-kynurenine pathway activity in this circuit causes cognitive dysfunction. This indicates a major potential role for the tryptophan-kynurenine enhancement in central fatigue, and these outcomes established the importance of the fatigue circuit.

In the future, it will be necessary to clarify the associations between central fatigue and neuroplasticity because tryptophan and its neuroactive metabolites are thought to play key roles in neuroplasticity. Given that increased tryptophan-kynurenine pathway activity from blood to brain is responsible for central fatigue, it is speculated that central fatigue carries an increased risk of glial and neuronal death. In fact, elevated tryptophan concentration suppresses neuron firing and elevated kynurenine metabolites take part in oligodendrocyte injury.

Finally, the basic findings in this review have the promise of novel insights into the consequences of central fatigue, for example, school refusal in children. Thus, this work may greatly contribute to elucidating latent mental problems in society from a scientific perspective.

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Author Contributions

MY conceived of and designed the experiments, analyzed the data, and wrote the first draft of the manuscript. The author reviewed and approved the final draft.

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