

## Tryptophan and Kynurenic Acid May Produce an Amplified Effect in Central Fatigue Induced by Chronic Sleep Disorder

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**ABSTRACT:** Tryptophan (TRP) and its neuroactive metabolite, kynurenic acid (KYNA), are thought to play key roles in central fatigue, but the specifics are still unknown. To clarify their roles in the brain, we developed a rat model of central fatigue induced by chronic sleep disorder (CFSD) by disturbing the sleep-wake cycle. Results showed that while 5-hydroxytryptamine (5-HT) concentration did not differ between control and CFSD groups, levels of TRP and KYNA in the CFSD group were about 2 and 5 times higher in the hypothalamus, and 2 and 3.5 times higher in the hippocampus, respectively. Moreover, CFSD-induced fatigue led to abnormal running performance (via treadmill test) and social interaction (via social-interaction test). These results support a TRP-KYNA hypothesis in central fatigue in which increased TRP concentration in the brain and subsequently synthesized KYNA may produce an amplified effect on central fatigue, with enhanced concentrations being a possible mechanism by which social-interaction deficits are generated.

**KEYWORDS:** 5-hydroxytryptamine, central fatigue,  $\beta$ -endorphin, kynurenic acid, sleep disorder, tryptophan

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### Introduction

Central fatigue is implicated in clinical conditions such as chronic fatigue syndrome, and leads to reduced cognitive function, disrupted social life, and impaired brain functions. In adults, these conditions can result in retirement or suspension from work. Similarly, the prevalence of central fatigue that is induced by chronic sleep disorders in schoolchildren has been reported at 40–80%.<sup>1,2</sup> Children are occasionally excused from school,<sup>3–5</sup> and brain function can become disrupted.<sup>1,2,4,6</sup>

Studies have reported that an increase in plasma concentration of free tryptophan (TRP) can result in postoperative or exercise-induced fatigue in human and rats.<sup>7–10</sup> This leads to increased passage of TRP in the brain through the blood-brain barrier (BBB) and thus higher levels of 5-hydroxytryptamine (5-HT) in the brain, which is theorized to cause central fatigue (5-HT hypothesis).<sup>7–10</sup> Kinn et al.<sup>11</sup> have reported that social behavior and sleep architecture are closely connected in anxiety- and depression-like symptoms following abnormally

poor quality of sleep. Very recently, the TRP-kynurenic acid (KYNA) hypothesis has been proposed to explain the mechanism of central fatigue.<sup>9,12</sup> However, no study has yet shown endogenous KYNA concentration in the brain fatigue. Moreover, neither the relationship between social behavior and central fatigue induced by chronic sleep disorder (CFSD) nor whether the TRP-KYNA hypothesis can account for the development of CFSD in a rat model is clear.

$\beta$ -Endorphin ( $\beta$ -EP) is a well-known suppressor of central fatigue,<sup>13</sup> and its synthesis is controlled by the hypothalamus.<sup>14,15</sup> Brain  $\beta$ -EP has been shown to alleviate excessive responses to psychological stress and fatigue<sup>16,17</sup> such as sleep disorders. While  $\beta$ -EP cannot generally pass through the BBB, previous animal studies have shown that the BBB can be disrupted by stress and fatigue.<sup>18,19</sup> Indeed, in the rat model of TRP-induced fatigue, peripheral administration of  $\beta$ -EP has been shown to restore an indicator of sympathetic nervous activity in urinary noradrenaline and 4-hydroxy-3-methoxyphenylglycol.<sup>13</sup>



The present study was designed to expose the relationship between social behavior and levels of TRP, KYNA, and 5-HT in the brain by using an animal model of central fatigue (CFSD rats<sup>20</sup>). In addition, to develop an effective treatment for recovery from central fatigue, we used CFSD rats to investigate the therapeutic properties of  $\beta$ -EP.

## Materials and Methods

**Animals.** This work was performed in accordance with guidelines provided by the Japanese Neuroscience Society for animal experiments, and was sanctioned by the Animal-Research Ethics Committee of Tezukayama University. Female Wistar rats (Japan SLC Inc., Hamamatsu, Japan,  $n = 15$ ) were housed individually under a 12-hour light-dark schedule (lights on at 8:00 am) in a humidity-controlled (55%) and temperature-controlled (22°C) colony room (CLEA Japan, Inc., Osaka, Japan). Seven-week-old rats weighing 100–120 g were used throughout all experiments, and were divided into a control group ( $n = 5$ ), CFSD group ( $n = 6$ ), and CFSD +  $\beta$ -EP treatment group ( $n = 4$ ). The rats had free access to food and water.

**Running performance.** During breeding, rats were trained to run using a treadmill (Japan SHINANO-SEISAKUSHO, SN-460) for seven days. Specifically, rats were first adapted to running on a treadmill for 15 minutes (maximum speed of 25 m/minute). The speed was gradually increased from 5 to 25 m/minute, and the duration from 15 to 60 minutes over the course of seven days. Additionally, while running, a weak current (below the 20 V) flowed from the electric stimulation zone at the end lane of the motorized treadmill. The electric stimulation was delivered to trigger motor running.

**Inducement of CFSD for the central fatigue model.** CFSD was induced using our methods previously described.<sup>20</sup> Briefly, rats were put in a plastic water tank (18.5 cm  $\times$  31.5 cm  $\times$  24.4 cm), fitted with a wooden refuge platform (6.5 cm  $\times$  5 cm). The tank was filled to 4.8 cm while the rats sat on the platform. Under these conditions, when rats lose muscle tone during rapid eye movement (REM) they fall into the water and wake up. Without enough REM sleep, they cannot get enough rest, and eventually develop CFSD. The CFSD model was generated in seven-week-old rats by depriving them of sleep for 20 hours/day for five days.

**$\beta$ -EP treatment.** Rat  $\beta$ -EP was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Rat  $\beta$ -EP (2.5  $\mu$ g/kg) was dissolved in 0.9% saline and injected i.p. half an hour before the end of the rest period during CFSD sleep-deprivation days 1, 3, and 5.

**Preparation of brain samples.** Rats (seven-weeks old) were sacrificed by decapitation at the end of behavioral experiments to determine the TRP metabolites in the brain. Brains were removed as quickly as possible. Dissections were performed on an ice-cooled aluminum plate. The brain was dissected into the following five areas: hippocampus, striatum,

hypothalamus, limbic system, and cerebellum.<sup>21</sup> These brain areas were transferred immediately to a polypropylene tube in 3% perchloric acid containing 1 mM  $\text{Na}_2\text{S}_2\text{O}_5$  0.05 g and 0.2% EDTA-2Na 0.5 g, and then homogenized with a polytron homogenizer for 10 seconds. Homogenized tissues were centrifuged at 10,000 rpm for 10 minutes at 4 °C. The supernatant was stored at –78 °C until conducting the assay for high performance liquid chromatography with electrochemical detector (HPLC-ECD, Irica, Japan) and fluorescence detector (HPLC-FLD, Nanospace SI-2 3001, Shiseido, Japan) systems.

**Separate determinations of TRP, 5-HT, 5-hydroxy-indole acetic acid (5-HIAA), and KYNA.** Concentrations of TRP, 5-HT, and 5-HIAA (Sigma-Aldrich Inc., Tokyo, Japan) were measured in each different brain region using HPLC-ECD and a chromate-recorder 12 (Irica, Japan). The temperature of the analytical octadecyl carbon chain (C18)-bonded silica columns (TSK gel, ODS-80 TM, 5  $\mu$ M, 4.6 mm i.d.  $\times$  15 cm, Tosoh, Japan) was maintained at 25 °C (Asone, Japan). The mobile phase was 15% methanol in a solution (pH 4.13) containing 30 mM citric acid, 10 mM  $\text{Na}_2\text{HPO}_4$ , 0.5 mM sodium octyl sulfate, 50 mM NaCl, and 0.05 mM EDTA, using methods previously described.<sup>7</sup> A flow rate of 0.7 mL/minute and an applied voltage of 700 (5-HT, 5-HIAA) or 800 mV (TRP) were employed. Frozen brain region homogenates were centrifuged at 4 °C for 10 minutes at 10,000 rpm (RA-150 AM, Kubota 1700, Japan). The supernatants were directly injected into the HPLC system.

KYNA (Wako Pure Chemical Industries Ltd., Osaka, Japan) concentration was measured in the hypothalamus, hippocampus, and striatum using HPLC-FLD as previously reported.<sup>22,23</sup> The HPLC system used for KYNA analysis consisted of the following: a FLD (Nanospace SI-3 3013, Shiseido, Japan) set at an excitation wave length of 344 nm and an emission wavelength of 398 nm, and a Shimadzu C-R8A chromate-recorder. The mobile phase consisted of 30 mM citric acid, 10 mM  $\text{Na}_2\text{HPO}_4$ , 0.5 mM octyl sodium sulfate, 50 mM NaCl, and 0.05 mM EDTA, and was pumped through a octadecyl carbon chain (C18)-bonded silica columns (TSK gel, ODS-80 TM, 5  $\mu$ M, 4.6 mm i.d.  $\times$  15 cm, Tosoh, Japan) at a flow rate of 1.0 mL/minute, and run at a temperature of 40 °C. Frozen brain region homogenates were centrifuged at 4 °C for 10 minutes at 10,000 rpm. The supernatants were directly injected into the HPLC system.

**Treadmill and social-interaction tests.** The treadmill test was conducted after establishing motor learning in rats. During sleep-disorder loading, fatigue level was measured once each day via the treadmill test for 15 minutes at a speed of 25 m/minute and an uphill inclination of 7°. Fatigue level was defined as the percentage of time spent running. Additionally, the electrical stimulation used during training was omitted.

To confirm the effect of social interaction on fatigue induced by chronic sleep disorder, experimental rats were placed with unfamiliar partner rats in a square wooden box (45 cm  $\times$  45 cm  $\times$  39 cm) and a stereotyped social-interaction

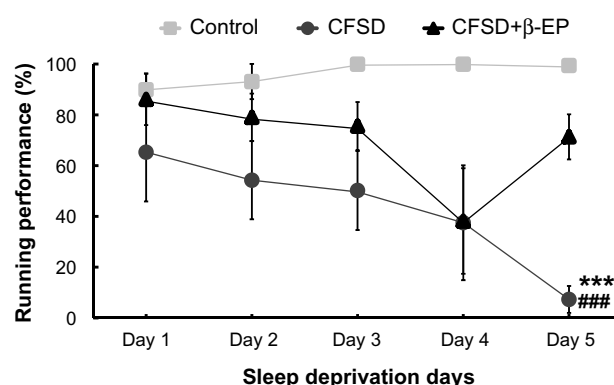
test was conducted using methods previously described.<sup>24–26</sup> Briefly, the total time (seconds) of sniffing, following, social grooming, and crawling over another rats (typical social interactions seen in rats) was observed for 10 minutes using a video-tracking camera (IVIS HF R21, Canon Inc., Tokyo, Japan). The treadmill test was conducted before the social interaction test.

**Statistical analyses.** The data from the treadmill and social-interaction tests were analyzed using two-way analyses of variance (ANOVA), followed by Bonferroni test for the simple main effects of rat group (control, CFSD, and CFSD +  $\beta$ -EP) and sleep-deprivation day (1–5). TRP, KYNA, 5-HT, and 5-HIAA concentrations in each different brain region were analyzed using a Student's *t*-test (control and CFSD groups).

## Results

**Sleep deprivation induced impairments in running performance and social interaction.** Performance ratios for the treadmill tests are provided in Figure 1. A two-way ANOVA of group (control, CFSD, and CFSD +  $\beta$ -EP) and sleep-deprivation day (1–5) showed a significant main effect of group ( $F[2, 8] = 6.59, P = 0.02$ ), sleep-deprivation day ( $F[3.88, 31.02] = 3.021, P = 0.034$ ), and the interaction (group  $\times$  deprivation day,  $F[7.75, 31.02] = 2.74, P = 0.022$ ). Closer analysis of the simple main effect revealed that while reduction in treadmill performance after sleep-deprivation day 3 was only marginally significant (control:  $99.9 \pm 0.04\%$  vs. CFSD:  $50.2 \pm 15.6\%$ ,  $P = 0.058$ ), reduction after day 5 (control:  $99.4 \pm 0.6\%$  vs. CFSD:  $7.3 \pm 5.3\%$ ,  $P < 0.001$ ) was both drastic and highly significant. Treatment with  $\beta$ -EP partially rescued the running deficit observed after day 5 (control [above] vs. CFSD +  $\beta$ -EP:  $71.3 \pm 8.9\%$ ,  $P = 0.059$ ; CFSD [above] vs. CFSD +  $\beta$ -EP [above],  $P < 0.001$ ).

Interaction times from the social-interaction tests are provided in Figure 2. A two-way ANOVA of group (control, CFSD, and CFSD +  $\beta$ -EP) and sleep-deprivation day (1–5) showed a significant main effect of group ( $F[2, 8] = 24.54, P < 0.001$ ), no significant main effect of sleep-deprivation day ( $F[4.00, 32.00] = 0.315, P = 0.87$ ), and a marginally significant interaction (group  $\times$  deprivation day:  $F[8.00, 32.00] = 1.99, P = 0.079$ ). Closer analysis of the simple main effect revealed reduction in social-interaction time after sleep-deprivation days 1 (control:  $90.8 \pm 8.6$  seconds vs. CFSD:  $26.8 \pm 3.8$  seconds,  $P < 0.001$ ), 2 (control:  $170.6 \pm 14.3$  seconds vs. CFSD:  $20.7 \pm 3.1$  seconds,  $P = 0.001$ ), and 4 (control:  $84.7 \pm 6.5$  seconds vs. CFSD:  $32.3 \pm 2.5$  seconds,  $P = 0.006$ ), and marginally shortened social-interaction time after sleep-deprivation day 3 (control:  $79.8 \pm 4.9$  seconds vs. CFSD:  $32.9 \pm 2.6$  seconds,  $P = 0.071$ ). Treatment with  $\beta$ -EP rescued the social-interaction deficit found in all days (day 1: CFSD [above] vs. CFSD +  $\beta$ -EP,  $61.8 \pm 6.5$  seconds,  $P = 0.009$ ; day 2: CFSD [above] vs. CFSD +  $\beta$ -EP,  $73.0 \pm 11.0$  seconds,  $P = 0.013$ ; day 3: CFSD [above] vs.

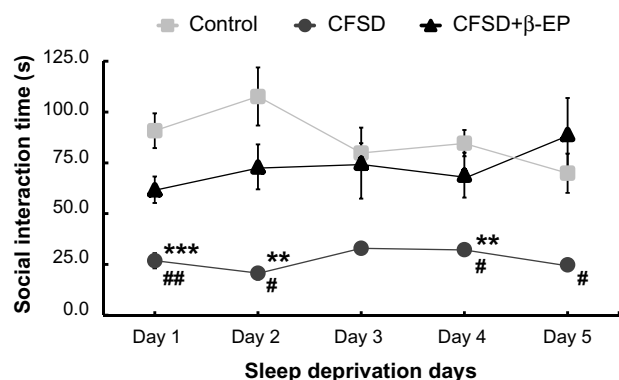


**Figure 1.** Effect of sleep disorder on running performance. Running performance in the treadmill test for control (■), CFSD (●), and CFSD +  $\beta$ -EP treatment (▲) groups on sleep-deprivation days 1–5. Parameters are expressed as mean  $\pm$  SEM. On sleep-deprivation day 3, CFSD marginally reduced treadmill performance (control = CFSD +  $\beta$ -EP  $\geq$  CFSD). On sleep-deprivation day 5, CFSD drastically reduced treadmill performance, and this deficit was partially rescued with  $\beta$ -EP treatment (control  $\geq$  CFSD +  $\beta$ -EP  $>$  CFSD).

**Notes:** Control versus CFSD. \*\*\* $P < 0.001$ , CFSD versus CFSD +  $\beta$ -EP. ### $P < 0.001$ , two-way ANOVA with Bonferroni test comparing CFSD, CFSD +  $\beta$ -EP, and control groups.

CFSD +  $\beta$ -EP,  $74.8 \pm 17.5$  seconds,  $P = 0.083$ ; day 4: CFSD [above] vs. CFSD +  $\beta$ -EP,  $68.9 \pm 11.1$  seconds,  $P = 0.026$ ; and day 5: CFSD [above] vs. CFSD +  $\beta$ -EP,  $89.0 \pm 17.9$  seconds,  $P = 0.015$ ). These results show that the fatigue observed in the animal model of CFSD is located centrally and that it led to abnormal social interaction.

**Sleep deprivation induced increases in TRP and KYNA concentrations in the hypothalamus and hippocampus.** To determine the TRP metabolites in the brain, rats were sacrificed by decapitation after sleep-deprivation day 5. The effect of sleep disturbance on TRP, 5-HT, and 5-HIAA concentrations in several areas of the brain is provided in Table 1 for control and CFSD groups. Compared to controls, CFSD rats exhibited significantly increased levels of TRP in the hypothalamus ( $t[4] = 5.29, P = 0.006$ ) and hippocampus ( $t[4] = 4.061, P = 0.015$ ), and marginally increased levels of TRP in the limbic system ( $t[4] = 2.77, P = 0.05$ ). Similarly, CFSD rats showed significantly increased levels of KYNA in the hypothalamus (Fig. 3: control,  $2.2 \pm 0.7$  nmol/g; CFSD,  $10.3 \pm 0.7$  nmol/g;  $t[9] = 8.4, P < 0.001$ ) and hippocampus (Fig. 3: control,  $2.1 \pm 0.6$  nmol/g; CFSD,  $7.8 \pm 1.3$  nmol/g;  $t[9] = 3.6, P = 0.006$ ). In contrast, 5-HT concentration decreased significantly in the striatum ( $t[4] = 2.96, P = 0.041$ ), hypothalamus ( $t[4] = 10.21, P = 0.001$ ), and cerebellum ( $t[4] = 3.55, P = 0.024$ ) of these rats. 5-HIAA concentrations marginally increased in the hypothalamus ( $t[4] = 2.47, P = 0.069$ ) and limbic system ( $t[4] = 2.57, P = 0.062$ ). These results show that TRP and KYNA concentrations were 2.5–5 times higher in the hypothalamus and hippocampus of the CFSD group compared to that in the control group.



**Figure 2.** Effect of sleep disorder on social interaction. Social-interaction time in the social-interaction test is shown by control (■), CFSD (●), and CFSD + β-EP treatment (▲) groups on sleep-deprivation days 1–5. Parameters are expressed as mean ± SEM. On sleep-deprivation day 1, CFSD drastically reduced interaction time, and this deficit was partially rescued with β-EP treatment (control = CFSD + β-EP > CFSD). On sleep-deprivation day 2, CFSD drastically reduced interaction time, and this deficit was partially rescued with β-EP treatment (control = CFSD + β-EP > CFSD). On sleep-deprivation day 3, CFSD marginally reduced interaction time, and this deficit was partially rescued with β-EP treatment (control = CFSD + β-EP ≥ CFSD). On sleep-deprivation day 4, CFSD drastically reduced interaction time, and this deficit was partially rescued with β-EP treatment (control = CFSD + β-EP > CFSD). On sleep-deprivation day 5, CFSD drastically reduced interaction time, and this deficit was partially rescued with β-EP treatment (control = CFSD + β-EP > CFSD).

**Notes:** Control versus CFSD. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , CFSD versus CFSD + β-EP. # $P < 0.05$ , ## $P < 0.01$ , two-way ANOVA with Bonferroni test comparing CFSD, CFSD + β-EP, and control groups.

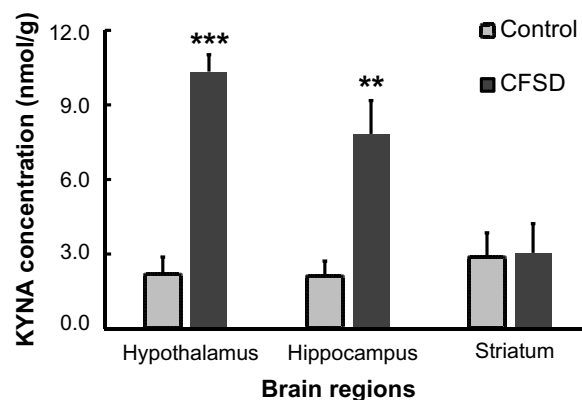
## Discussion

Studies have reported that an increase in plasma concentration of free TRP can result in postoperative or exercise-induced fatigue in human and rats, respectively.<sup>7–10</sup> Additionally, more

**Table 1.** Effect of biological rhythm disturbance on the concentrations (nmol/g) of TRP, 5-HT, and 5-HIAA in several regions of the brain for control and CFSD rats.

BRAIN REGIONS	GROUP	TRYPTOPHAN METABOLITE CONCENTRATIONS [NMOL/G]		
		TRP	5-HT	5-HIAA
Hypothalamus	Control	22.6 ± 2.2	16.6 ± 0.6	11.6 ± 4.1
	CFSD	53.0 ± 5.3**	6.3 ± 0.8**	25.1 ± 3.7
Hippocampus	Control	13.0 ± 1.0	8.3 ± 2.2	12.5 ± 1.3
	CFSD	26.0 ± 3.0*	6.5 ± 2.6	19.2 ± 5.3
Limbic system	Control	11.8 ± 2.1	6.7 ± 1.7	7.5 ± 2.2
	CFSD	29.6 ± 6.1	5.9 ± 2.6	14.9 ± 1.9
Striatum	Control	22.5 ± 3.2	9.2 ± 2.4	12.0 ± 1.6
	CFSD	27.2 ± 4.0	2.0 ± 0.5*	16.2 ± 2.1
Cerebellum	Control	9.1 ± 2.3	0.6 ± 0.1	1.0 ± 0.4
	CFSD	9.7 ± 1.3	0.4 ± 0.01*	1.0 ± 0.01

**Notes:** Parameters are expressed as mean ± SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , Student's *t*-test compared to the control group.



**Figure 3.** KYNA concentration in several regions of the brain for control and CFSD rats. KYNA concentration in the hypothalamus and hippocampus significantly increased in the CFSD rats compared to controls, whereas no change was observed in the striatum (control: 2.9 ± 1.0 nmol/g, CFSD: 3.0 ± 1.2 nmol/g). Parameters are expressed as mean ± SEM.

**Notes:** \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , Student's *t*-test compared to the control group.

free TRP crosses the BBB in the brain, and leads to higher levels of 5-HT.<sup>7–10,27,28</sup> However, because an animal model of central fatigue has not yet been generated, we did so here using CFSD. TRP concentration in the hippocampus and hypothalamus drastically increased in the CFSD rats compared to that in the controls, whereas no change was seen in motor-system areas such as the striatum or cerebellum (Table 1). While TRP concentration in hippocampal and hypothalamic synaptosomes corresponded well with reports regarding a rat model of central fatigue that employed a treadmill,<sup>27</sup> the invariant TRP concentration that we found in the striatum and cerebellum in the CFSD rats did not correspond with previous reports.<sup>7,10,27</sup> Thus, the CFSD generates central fatigue that leads to an increase in TRP concentration specifically by the hippocampus and hypothalamus. Further, CFSD did not induce increases in 5-HT concentration in any of the five brain regions we examined (Table 1). According to the 5-HT hypothesis of central fatigue, 5-HT synthesis rises with increased transport of TRP into those brain regions.<sup>7–10,27,28</sup> This theory is belied by our results showing increased levels of TRP in the hippocampus and hypothalamus of CFSD rats, but no similar increase in 5-HT synthesis.

Our results can define a key role of the TRP-KYNA pathway in behavioral suppression and dysfunction seen in central fatigue. In mammals, outside of 5-HT synthesis, the vast majority of TRP is metabolized via the kynurenine pathway into KYNA and quinolinic acid (QUIN).<sup>29</sup> While QUIN is an *N*-methyl-D-aspartic acid (NMDA) receptor agonist, KYNA has also been reported as an antagonist of both NMDA and α-7 nicotinic acetylcholine (α7nACh) receptors.<sup>29,30</sup> Therefore, KYNA is considered to take part in glutamatergic and cholinergic neurotransmission in the central nervous system. Previous reports have shown that an increase in KYNA in the central



nervous system reduces glutamatergic neurotransmission.<sup>23,29,31</sup> Very recently, injection of KYNA was shown to impair rat performance in the running, open-field, and Morris water-maze tests.<sup>12</sup> This indicated that central fatigue could be caused by KYNA, but whether endogenous brain KYNA causes central fatigue remained unclear. We therefore measured KYNA concentration in the hypothalamus, hippocampus, and striatum. KYNA and TRP concentrations in the hypothalamus and hippocampus drastically increased in the CFSD rats compared to controls (Fig. 3), whereas no change was seen in the striatum (Fig. 3). These data have shown that CFSD led to an increase in concentration of TRP and subsequently synthesized KYNA specifically in the hypothalamus and hippocampus. The hippocampus and hypothalamus subserve memory-learning, social memory, social experience, and social behavior.<sup>32–34</sup> Electrophysiologically, increased TRP has been found to inhibit the firing of raphe neurons.<sup>35</sup> Therefore, higher levels of TRP in our study (Table 1) may have suppressed neuronal firing in the hypothalamus and hippocampus. Moreover, pharmacologically, increased KYNA levels in the brain cause inhibition of  $\alpha 7$ nACh and NMDA receptors, and a secondary reduction in glutamate levels.<sup>29,31,36</sup> Reduction in glutamate levels has been implicated in cognitive and social impairment-associated memory loss,<sup>37</sup> poor treadmill performance,<sup>12</sup> and impaired social behavior.<sup>38</sup> Therefore, as these receptors are at least involved in hypothalamic processing, higher levels of KYNA in our study (Fig. 3) and the associated reduction in glutamate levels may underlie the neurocognitive dysfunction in social interaction (Fig. 2) and psychomotor activity (Fig. 1) seen in our CFSD model. Thus, this suggests that TRP and KYNA may produce an amplified effect in central fatigue. The role of KYNA in fatigue that we report is supported by the recent findings<sup>39</sup> that administration of a branched-chain amino acid that lowers exercise-induced fatigue also reduces the higher levels of KYNA in the brain. Moreover, it has been reported that exogenous KYNA increases fatigability and the administration of KYNA into the hippocampus decreases neurocognition.<sup>12</sup> Here we provide the first evidence that both endogenous brain KYNA and TRP increase in central fatigue. The mechanism may derive from activation of indoleamine-2,3-dioxygenase in the brain, which leads to increased plasma-free TRP and kynurenine uptake into the brain, which is subsequently used to synthesize KYNA.

Although comparisons between control and CFSD groups during sleep-deprivation days 1–2 (acute stage) did not show significant differences in running performance (Fig. 1), social-interaction tests did reveal significant adverse changes during that period (Fig. 2), indicating that our central-fatigue model likely generates social-interaction failure. The CFSD group expressed both central fatigue (Fig. 1) and social-interaction failure (Fig. 2) during sleep-deprivation days 3 (sub-acute stage) and 5 (chronic stage). CFSD led to complete exhaustion, and recovery through rest in the home cage was difficult without  $\beta$ -EP treatment to block central fatigue.

Here, we developed an effective treatment for recovery from CFSD.  $\beta$ -EP was administered on sleep-deprivation days 1, 3, and 5, and proved to effectively reverse CFSD-induced lack of motivation, social-interaction deficits, and emotional upset (Figs. 1 and 2). Thus,  $\beta$ -EP might increase social-motivation. Psychological stress and fatigue increases the permeability of the BBB,<sup>18,19</sup> explaining the higher TRP levels throughout a wide region of the brain in CFSD rats (Table 1), and also allows peripherally administered  $\beta$ -EP to enter the brain. These findings suggest that  $\beta$ -EP may suppress the effect of increased TRP in the brain. Specifically,  $\beta$ -EP derived from the circulation may act in areas of the limbic-hypothalamic circuit such as the amygdaloid nuclei. It may also act to raise the threshold of exhaustion, and could thus be useful for its alleviation. Indeed, it reversed the adverse effects of CFSD. However, the relationship between endogenous TRP-KYNA concentrations and the pharmacological effect of  $\beta$ -EP in central fatigue remains to be explored further. As  $\beta$ -EP is known to suppress central fatigue, thus, our CFSD model can be said to induce central fatigue. Further support for this claim is seen in the increase in TRP levels in the CFSD rats. Our results provide the first evidence that an amplified effect exists when both TRP and KYNA increase. Finally, because the central-fatigue model is similar to CFSD, and the pathological characteristics induced by childhood chronic fatigue syndrome,<sup>1–6</sup> we expect that this model will help to resolve the mechanism of central fatigue in schoolchildren induced by chronic sleep disorder.

## Conclusion

The present findings indicate a potential role of endogenous KYNA in central fatigue, and demonstrate that central fatigue can be caused by altered TRP concentration in the brain. The results also show that excessive levels of TRP lead to enhanced KYNA synthesis, but not to enhanced 5-HT synthesis. Thus, increased TRP concentration in the brain and subsequently synthesized KYNA may produce an amplified effect that induces central fatigue and possibly directly lead to social-interaction deficits. Furthermore, because  $\beta$ -EP reduced the observed social-interaction deficit and acts centrally,  $\beta$ -EP may be useful for prevention and recovery from central fatigue.

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## Author Contributions

Conceived and designed the experiments, analyzed the data, and wrote the first draft of the manuscript: MY. Conceived research hypotheses and ideas: TY. Contributed to conduct-



ing methods of HPLC assay: TY. Contributed to the writing of the manuscript, agreed with manuscript results and conclusions, jointly developed the structure and arguments for the paper, and made critical revisions and approved the final version: MY, TY. All authors reviewed and approved the final manuscript.

## DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copy-righted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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