

( $F(5, 52) = 17.358, P < 0.0001$ ) significantly altered the dependent measures. The interactions between ageing and housing condition ( $F(5, 52) = 7.624, P < 0.0001$ ) and between housing condition and novelty stress ( $F(5, 52) = 7.872, P < 0.0001$ ) were significant, whereas the interaction between ageing and novelty stress ( $F(5, 52) = 1.894, P = 0.11$ ) was not significant. The interaction among ageing, housing condition and novelty stress ( $F(5, 52) = 5.046, P = 0.0008$ ) was significant.

The *post hoc* tests revealed that ageing significantly decreased TRP concentration ( $p < 0.01$ ), whereas it increased the 5-HT concentration ( $p < 0.01$ ) and the 5-HT/TRP ratio ( $p < 0.05$ , Figure 2A, Table IA).

**Young adult group.** The results of two-way MANOVA for housing condition and novelty stress were as follows: housing condition ( $F(24, 5) = 30.209, P < 0.0001$ ) and novelty stress ( $F(24, 5) = 21.698, P < 0.0001$ ) significantly altered the dependent measures. The interaction between housing condition and novelty stress ( $F(24, 5) = 16.958, P < 0.0001$ ) was significant.

The *post hoc* tests revealed that social isolation significantly decreased TRP ( $p < 0.01$ ) and KYN ( $p < 0.05$ ) levels, whereas it increased the KYN/TRP ( $p < 0.05$ ) and 5-HT/TRP ( $p < 0.01$ ) ratios (Figure 2A, Table IA). Novelty stress significantly increased the TRP ( $p < 0.01$ ) and KYN ( $p < 0.05$ ) levels, and decreased the 5-HT/TRP ratio ( $p < 0.01$ , Figure 2A, Table IA).

**Adult group.** The results of two-way MANOVA for housing condition and novelty stress were as follows: housing condition ( $F(24, 5) = 3.318, P = 0.02$ ) and novelty stress ( $F(24, 5) = 4.075, P = 0.008$ ) significantly altered the dependent measures. The interaction between housing condition and novelty stress ( $F(24, 5) = 1.874, P = 0.14$ ) was not significant.

The *post hoc* tests revealed that social isolation significantly increased the 5-HT level ( $p < 0.01$ ) and 5-HT/TRP ratio ( $p < 0.01$ , Figure 2A, Table IA). Novelty stress significantly increased the TRP level ( $p < 0.01$ , Figure 2A, Table IA).

**Summary.** Ageing decreased the TRP concentration and increased the 5-HT concentration, thus increasing the 5-HT/TRP ratio in the prefrontal cortex. Ageing shifted the balance between the KYN and 5-HT pathways to the latter. In the younger group, novelty stress increased both the TRP and KYN levels, but decreased the 5-HT/TRP ratio. Thus, novelty stress shifted the balance between the KYN and 5-HT pathways to the former, although the stress did not directly attenuate the 5-HT level. Social

isolation decreased the TRP and KYN levels but increased the KYN/TRP and 5-HT/TRP ratios. In the older group, novelty stress increased the TRP level, whereas it altered neither the KYN nor 5-HT level. Thus, novelty stress did not shift the balance between the KYN and 5-HT pathways. Social isolation increased the 5-HT level and 5-HT/TRP ratio. Hence, social isolation shifted the balance between the KYN and 5-HT pathways to the latter.

#### Hippocampus

**Ageing, housing condition, and novelty stress.** Ageing ( $F(5, 52) = 9.240, P < 0.0001$ ), housing condition ( $F(5, 52) = 8.596, P < 0.0001$ ) and novelty stress ( $F(5, 52) = 13.178, P < 0.0001$ ) significantly altered the dependent measures. The interactions between ageing and housing condition ( $F(5, 52) = 4.025, P = 0.004$ ), between ageing and novelty stress ( $F(5, 52) = 4.613, P = 0.001$ ) and between housing condition and novelty stress ( $F(5, 52) = 2.423, P = 0.048$ ) were significant. The interaction among ageing, housing condition and novelty stress ( $F(5, 52) = 3.508, P = 0.008$ ) was significant.

The *post hoc* tests revealed that ageing significantly decreased the TRP level ( $p < 0.01$ ) but increased the 5-HT level ( $p < 0.01$ ) and the 5-HT/TRP ratio ( $p < 0.01$ , Figure 2B, Table IB).

**Young adult group.** The results of two-way MANOVA for housing condition and novelty stress were as follows: housing condition ( $F(24, 5) = 14.800, P < 0.0001$ ) and novelty stress ( $F(24, 5) = 14.210, P < 0.0001$ ) significantly altered dependent measures. The interaction between housing condition and novelty stress ( $F(24, 5) = 11.194, P < 0.0001$ ) was significant.

The *post hoc* tests revealed that social isolation significantly decreased the TRP ( $p < 0.01$ ) level and increased the 5-HT/TRP ( $p < 0.01$ ) ratio (Figure 2B, Table IB). Novelty stress significantly increased the TRP ( $p < 0.01$ ) and KYN ( $p < 0.05$ ) levels and decreased the 5-HT/TRP ratio ( $p < 0.01$ , Figure 2B, Table IB).

**Adult group.** The results of two-way MANOVA for housing condition and novelty stress were as follows: housing condition ( $F(24, 5) = 1.208, P = 0.33$ ) did not significantly alter the dependent measures, whereas novelty stress ( $F(24, 5) = 7.063, P = 0.0003$ ) did. The interaction between housing condition and novelty stress ( $F(24, 5) = 0.826, P = 0.54$ ) was not significant. The *post hoc* test revealed that novelty stress significantly increased the TRP ( $p < 0.01$ ) level and decreased the KYN/TRP ( $p < 0.05$ ) ratio (Figure 2B, Table IB).

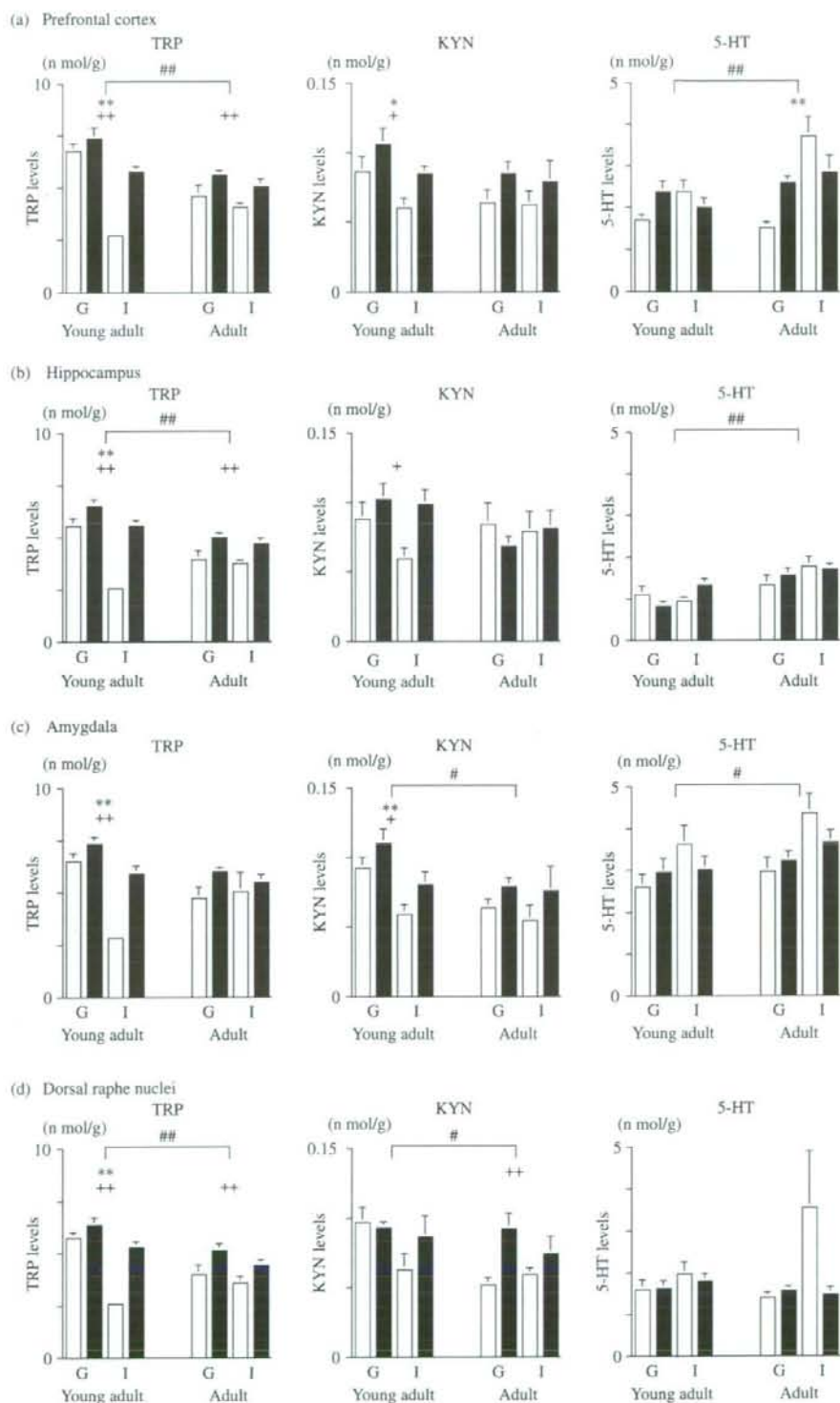


Table I. Changes in the KYN/TRP and 5-HT/TRP ratios elicited by aging, housing condition and novelty stress.

(a) Prefrontal cortex			KYN/TRP		5-HT/TRP	
<i>Young adult</i>						
Group-housing	Stress (-)		0.013 ± 0.002		0.254 ± 0.022	
	Stress (+)		0.015 ± 0.002		0.339 ± 0.053	
Isolation-housing	Stress (-)		0.023 ± 0.003		0.898 ± 0.108	
	Stress (+)		0.015 ± 0.001	*	0.347 ± 0.037	**
<i>Adult</i>						
Group-housing	Stress (-)		0.015 ± 0.002		0.371 ± 0.057	#
	Stress (+)		0.015 ± 0.001		0.466 ± 0.029	
Isolation-housing	Stress (-)		0.015 ± 0.003		0.927 ± 0.115	
	Stress (+)		0.016 ± 0.004		0.580 ± 0.098	**++
(b) Hippocampus						
<i>Young adult</i>						
Group-housing	Stress (-)		0.017 ± 0.003		0.200 ± 0.031	
	Stress (+)		0.016 ± 0.002		0.131 ± 0.019	
Isolation-housing	Stress (-)		0.024 ± 0.003		0.383 ± 0.039	
	Stress (+)		0.017 ± 0.002		0.240 ± 0.028	**++
<i>Adult</i>						
Group-housing	Stress (-)		0.021 ± 0.002		0.364 ± 0.069	##
	Stress (+)		0.013 ± 0.001		0.313 ± 0.030	
Isolation-housing	Stress (-)		0.021 ± 0.003		0.472 ± 0.055	
	Stress (+)		0.017 ± 0.003	+	0.368 ± 0.027	
(c) Amygdala						
<i>Young adult</i>						
Group-housing	Stress (-)		0.014 ± 0.001		0.401 ± 0.045	
	Stress (+)		0.015 ± 0.002		0.401 ± 0.042	
Isolation-housing	Stress (-)		0.021 ± 0.003		1.319 ± 0.190	
	Stress (+)		0.014 ± 0.002		0.526 ± 0.055	**++
<i>Adult</i>						
Group-housing	Stress (-)		0.015 ± 0.002		0.690 ± 0.105	
	Stress (+)		0.013 ± 0.001		0.542 ± 0.054	
Isolation-housing	Stress (-)		0.011 ± 0.001		1.014 ± 0.156	
	Stress (+)		0.014 ± 0.004		0.683 ± 0.054	+
(d) Dorsal raphe nuclei						
<i>Young adult</i>						
Group-housing	Stress (-)		0.017 ± 0.001		0.285 ± 0.036	
	Stress (+)		0.015 ± 0.001		0.285 ± 0.028	
Isolation-housing	Stress (-)		0.024 ± 0.004		0.758 ± 0.095	
	Stress (+)		0.017 ± 0.004		0.343 ± 0.034	**++
<i>Adult</i>						
Group-housing	Stress (-)		0.013 ± 0.002		0.378 ± 0.057	
	Stress (+)		0.018 ± 0.001		0.312 ± 0.023	
Isolation-housing	Stress (-)		0.017 ± 0.001		0.908 ± 0.242	
	Stress (+)		0.016 ± 0.004		0.332 ± 0.033	+

The number of mice used for each group (as defined by age, stress and housing condition) was 8. Values are shown as means ± SEM. The results of Tukey-Kramer test for aging, housing condition, and novelty stress are shown. Between young adult and adult groups, effects of ageing are shown: #,  $p < 0.05$ ; ##,  $p < 0.01$ . For each age group, effects of housing condition are shown: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ . Effects of novelty stress are shown: +,  $p < 0.05$ ; ++,  $p < 0.01$ . A, prefrontal cortex; B, hippocampus; C, amygdala; D, dorsal raphe nuclei.

**Summary.** Ageing decreased the TRP concentration and increased the 5-HT concentration, thus increasing the 5-HT/TRP ratio in the hippocampus. Ageing shifted the balance between the KYN and 5-HT pathways to the latter. In the younger group, novelty stress increased the TRP and KYN levels and

decreased the 5-HT/TRP ratio. Thus, novelty stress shifted the balance between the KYN and 5-HT pathways to the former, although the stress did not directly attenuate the 5-HT level. Social isolation decreased the TRP level and increased the 5-HT/TRP ratio. Thus, social isolation shifted the balance

Figure 2. Changes in the TRP, KYN and serotonin (5-HT) concentrations elicited by ageing, housing condition, and novelty stress. Each bar indicates a group defined by age, stress and housing condition ( $n = 8$ ). White bar, non-stress; black bar, stress. G, group housing; I, isolation housing. Values are shown as means ± SEM. The results of Tukey-Kramer test for ageing, housing condition, and novelty stress are shown. Between the young adult and adult groups, effects of ageing are shown: #,  $p < 0.05$ ; ##,  $p < 0.01$ . For each age group, effects of housing condition are shown: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ . Effects of novelty stress are shown: +,  $p < 0.05$ ; ++,  $p < 0.01$ . A, prefrontal cortex; B, hippocampus; C, amygdala; D, dorsal raphe nuclei.



between the KYN and 5-HT pathways to the latter. In the older group, novelty stress increased the TRP level and decreased the KYN/TRP ratio. Thus, novelty stress shifted the balance between the KYN and 5-HT pathways to the latter, although the stress did not directly attenuate the KYN level.

#### Amygdala

*Ageing, housing condition, and novelty stress.* Ageing ( $F(5, 52) = 3.580, P = 0.007$ ), housing condition ( $F(5, 52) = 8.778, P < 0.0001$ ) and novelty stress ( $F(5, 52) = 11.030, P < 0.0001$ ) significantly altered the dependent measures. The interactions between ageing and housing condition ( $F(5, 52) = 6.826, P < 0.0001$ ) and between housing condition and novelty stress ( $F(5, 52) = 3.553, P = 0.008$ ) were significant, whereas the interaction between ageing and novelty stress ( $F(5, 52) = 1.214, P = 0.32$ ) was not significant. The interaction among ageing, housing condition and novelty stress ( $F(5, 52) = 4.104, P = 0.003$ ) was significant.

The *post hoc* tests revealed that ageing significantly decreased the KYN concentration ( $p < 0.05$ ), whereas it increased the 5-HT concentration ( $p < 0.05$ , Figure 2C).

*Young adult group.* The results of two-way MANOVA for housing condition and novelty stress were as follows: housing condition ( $F(24, 5) = 18.549, P < 0.0001$ ) and novelty stress ( $F(24, 5) = 11.199, P < 0.0001$ ) significantly altered the dependent measures. The interaction between housing condition and novelty stress ( $F(24, 5) = 6.055, P = 0.0009$ ) was significant.

The *post hoc* tests revealed that social isolation significantly decreased the TRP ( $p < 0.01$ ) and KYN ( $p < 0.01$ ) levels but increased the 5-HT/TRP ( $p < 0.01$ ) ratio (Figure 2C, Table IC). Novelty stress significantly increased the TRP ( $p < 0.01$ ) and KYN ( $p < 0.05$ ) concentrations but decreased the 5-HT/TRP ratio ( $p < 0.01$ , Figure 2C, Table IC).

*Adult group.* The results of two-way MANOVA for housing condition and novelty stress were as follows: housing condition ( $F(24, 5) = 1.654, P = 0.18$ ) did not significantly alter the dependent measures, whereas novelty stress ( $F(24, 5) = 4.407, P = 0.005$ ) did. The interaction between housing condition and novelty stress ( $F(24, 5) = 1.444, P = 0.24$ ) was not significant. The *post hoc* test revealed that novelty stress significantly decreased the 5-HT/TRP ratio ( $p < 0.05$ , Table IC).

*Summary.* Ageing decreased the KYN concentration and increased the 5-HT concentration in the amygdala. Thus, ageing shifted the balance between the KYN and 5-HT pathways to the latter. In the younger group, novelty stress increased the TRP and KYN levels and decreased the 5-HT/TRP ratio. Thus, novelty stress shifted the balance between the KYN and 5-HT pathways to the former, although the stress did not directly attenuate the 5-HT level. Social isolation decreased the TRP and KYN levels, and increased the 5-HT/TRP ratio. Thus, social isolation shifted the balance between the KYN and 5-HT pathways to the latter. In the older group, novelty stress decreased the 5-HT/TRP ratio. Hence, novelty stress shifted the balance between the KYN and 5-HT pathways to the former, although the stress did not directly elevate KYN concentration.

#### Dorsal raphe nuclei

*Ageing, housing condition, and novelty stress.* Ageing ( $F(5, 52) = 3.851, P = 0.005$ ), housing condition ( $F(5, 52) = 13.107, P < 0.0001$ ) and novelty stress ( $F(5, 52) = 13.705, P < 0.0001$ ) significantly altered the dependent measures. The interactions between ageing and housing condition ( $F(5, 52) = 3.384, P = 0.01$ ) and between housing condition and novelty stress ( $F(5, 52) = 5.963, P = 0.0002$ ) were significant, whereas the interaction between ageing and novelty stress ( $F(5, 52) = 1.130, P = 0.35$ ) was not significant. The interaction among ageing, housing condition and novelty stress ( $F(5, 52) = 3.191, P = 0.001$ ) was significant.

The *post hoc* tests revealed that ageing significantly decreased the TRP ( $p < 0.01$ ) and KYN ( $p < 0.05$ ) concentrations (Figure 2D).

*Young adult group.* The results of two-way MANOVA for housing condition and novelty stress were as follows: housing condition ( $F(24, 5) = 20.183, P < 0.0001$ ) and novelty stress ( $F(24, 5) = 12.847, P < 0.0001$ ) significantly altered the dependent measures. The interaction between housing condition and novelty stress ( $F(24, 5) = 9.851, P < 0.0001$ ) was significant.

The *post hoc* tests revealed that social isolation significantly decreased the TRP ( $p < 0.01$ ) concentration and increased the 5-HT/TRP ( $p < 0.01$ ) ratio (Figure 2D, Table ID). Novelty stress significantly increased the TRP ( $p < 0.01$ ) level and decreased the 5-HT/TRP ratio ( $p < 0.01$ , Figure 2D, Table ID).

*Adult group.* The results of two-way MANOVA for housing condition and novelty stress were as follows: housing condition ( $F(24, 5) = 1.634, P = 0.19$ ) did not significantly alter the dependent measures,



whereas novelty stress ( $F(24, 5) = 4.778, P = 0.004$ ) did. The interaction between housing condition and novelty stress ( $F(24, 5) = 1.709, P = 0.17$ ) was not significant.

The *post hoc* tests revealed that novelty stress significantly increased TRP ( $p < 0.01$ ) and KYN ( $p < 0.01$ ) concentrations and decreased the 5-HT/TRP ( $p < 0.05$ ) ratio (Figure 2D, Table ID).

**Summary.** Ageing decreased the TRP and KYN levels in the dorsal raphe nuclei. In the younger group, novelty stress increased the TRP concentration and decreased the 5-HT/TRP ratio. Thus, novelty stress shifted the balance between the KYN and 5-HT pathways to the former, although the stress did not directly attenuate the 5-HT level. Social isolation decreased the TRP level and increased the 5-HT/TRP ratio. Thus, social isolation shifted the balance between the KYN and 5-HT pathways to the latter. In the older group, novelty stress increased the TRP and KYN levels and decreased the 5-HT/TRP ratio. Hence, novelty stress shifted the balance between the KYN and 5-HT pathways to the former, although the stress did not directly attenuate the 5-HT level.

**Overview.** Ageing shifted the balance between the KYN and 5-HT pathways to the latter. In the younger group, social isolation shifted the balance to the latter, whereas novelty stress shifted it to the former. In the older group, effects of social isolation and novelty stress were restricted to specific brain regions. Ageing and social isolation counteracted novelty stress effects on TRP metabolism.

## Discussion

Recent studies have suggested that changes in the balance between the activity of the KYN pathway and that of the 5-HT pathway in TRP metabolism play an important role in depression (Wichers et al. 2005; Myint et al. 2007). Here, we investigated changes in KYN system activity elicited by ageing, social environment and psychological stress to clarify whether acute psychological stress shifts the balance of activities between the KYN and 5-HT pathways to the former, and how ageing and social environment modulate the shifting of TRP metabolism.

### Effects of ageing on tryptophan metabolism

Ageing decreased TRP levels, decreased KYN levels, increased 5-HT levels and increased the 5-HT/TRP ratio in the brain regions studied here. Thus, ageing shifted the balance between the KYN and 5-HT pathways to the latter. Furthermore, ageing diminished TRP and KYN levels without altering the

KYN/TRP ratio. Previous studies have suggested that ageing activates the KYN pathway especially in KYN synthesis (Gramsbergen et al. 1992). In a human study, Alzheimer's disease patients and age-matched controls exhibited a decrease in plasma TRP level and an increase in KYN/TRP ratio as compared to the younger control group, and these changes were more prominent in the Alzheimer's disease group than in the age-matched controls (Widner et al. 2000). Our results partly confirmed these studies of ageing effects.

In the present study, ageing increased the brain 5-HT level; while some previous studies have reported an increase (Santiago et al. 1988; Delion et al. 1997), no change (Ponzio et al. 1982; Lee et al. 1994) or a decrease (Gozlan et al. 1990; Luine et al. 1990). Thus, the influence of ageing on brain 5-HT levels remains controversial. Our previous study using rats showed a decrease in the 5-hydroxyindoleacetic acid (5-HIAA)/5-HT ratio, or the 5-HT turnover ratio, elicited by ageing (Miura et al. 2002b). Evidently, the decreased 5-HT turnover elicited by ageing may have been the cause of the increased 5-HT level. To evaluate the effect of ageing on TRP metabolism especially in altering the 5-HT pathway, the age of 6 months studied in our protocol may have not been sufficient, although mice are usually retired from breeding at this age.

### Effects of social isolation and novelty stress on tryptophan metabolism, and the interaction between the two factors

**Young adult group.** Novelty stress increased brain TRP and KYN levels, whereas this stress did not change the 5-HT level. Although novelty stress left the KYN/TRP ratio unchanged, it decreased the 5-HT/TRP ratio. Hence, novelty stress shifted the balance between the KYN and 5-HT pathways to the former. Although social isolation left the brain KYN/TRP ratio unchanged, except for an increase in the prefrontal cortex, it increased the 5-HT/TRP ratio. Thus, social isolation shifted the balance between the KYN and 5-HT pathways to the latter.

Novelty stress may increase the brain TRP level by stimulation of the sympathetic nervous system. Plasma-free fatty acids (FFA) displace TRP bound to serum albumin, leading to an increase in the free concentration of TRP, which competes with so-called "large neutral" amino acids (LNAA) for active transport through the blood-brain barrier (Curzon et al. 1973). Lipolysis and FFA generation, as a result of sympathetic activation during stress, may have caused an elevation of free circulating TRP and consequently increased TRP transfer across the blood-brain barrier into the brain. In a human study, exercise decreased plasma LNAA and increased plasma free TRP concentrations and increased the free TRP/LNAA ratio (Struder et al. 1999). Thus, stress increased free plasma TRP, decreased LNAA



and then may have increased the brain TRP level. In an animal study, the physiological stress of foot shock increased brain TRP as well as KYN levels of rats (Pawlak et al. 2000). These changes elicited by foot shock were similar to our results indicating an elevation of brain TRP and KYN levels by novelty stress. Thus, brain IDO activity is likely to be present in physiological conditions without immunological challenge. Other studies indicate IDO activity in neurons as well as in microglia and astrocytes (Guillemin et al. 2004; Roy et al. 2005). The results suggest that brain IDO activity present in the normal physiological conditions can metabolise the increased TRP level elicited by novelty stress into KYN. These changes may not have induced IDO as much as may occur with immunological challenge. Consequently, the stress did not alter the KYN/TRP ratio.

**Adult group.** Novelty stress increased brain TRP level, but did not change 5-HT level. Although novelty stress left the KYN/TRP ratio unchanged, it decreased the 5-HT/TRP ratio. Thus, novelty stress shifted the balance between the KYN and 5-HT pathways to the former. However, novelty stress decreased the KYN/TRP ratio in the hippocampus. Only in the hippocampus, novelty stress shifted the balance between the KYN and 5-HT pathways to the latter, although the stress shifted the balance to the former in the younger group. The significance of the opposite effect of novelty stress on the balance between the KYN and 5-HT pathways in the hippocampus of the older group is unclear, although this might be relevant to the etiology and pathophysiology of depression. Social isolation shifted the balance between the KYN and 5-HT pathways to the former, but only in the prefrontal cortex.

## Summary

Ageing, social isolation and novelty stress, etiological risk factors of depression, did contribute to changes in TRP metabolism hypothesised as a pathophysiological mechanism of depression. However, ageing and social isolation counteracted novelty stress in the direction of shifting the balance between the KYN and 5-HT pathways. These results did not meet our expectation that the three risk factors would synergistically alter TRP metabolism. In the etiology of depression, the role of novelty stress in counteracting other factors remain unknown. Furthermore, the changes were slight as compared with those previously shown to be elicited by direct immunological stimulation, such as by proinflammatory cytokines. In particular, the three etiological risk factors studied here failed to reduce the brain 5-HT level. We suppose that our results indicate prolonged adaptation to these risk factors rather than the

physiological condition of depression itself. To produce the conditions assumed to represent depression in an animal model, further breakdown of the prolonged adaptation may be needed, because prolonged adaptation frequently precedes the clinical onset of human depression. Prolonged adaptation to these factors may compromise the availability of protection against new stressors on the body and brain. Such cumulative changes elicited by stress have been termed "allostatic load or overload" (McEwen 2004). Hence, prolonged adaptation can be considered the precondition that increases vulnerability. Novel activation of IDO by immunological challenge or other stressors might further modify the previously shifted balance between the KYN and 5-HT pathways elicited by these risk factors and result in the onset of disease.

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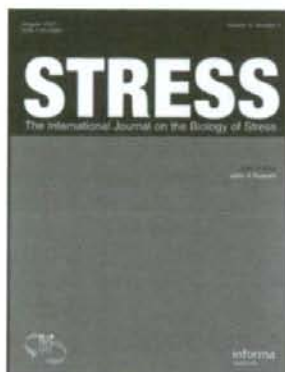
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## Stress

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### A link between stress and depression: Shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression

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## A link between stress and depression: Shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression

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### Abstract

Alteration of tryptophan (TRP) metabolism elicited by proinflammatory cytokines has gained attention as a new concept to explain the etiological and pathophysiological mechanisms of major depression. The kynurenine (KYN) pathway, which is initiated by indoleamine 2,3-dioxygenase (IDO), is the main TRP metabolic pathway. It shares TRP with the serotonin (5-HT) pathway. Proinflammatory cytokines induce IDO under stress, promote the KYN pathway, deprive the 5-HT pathway of TRP, and reduce 5-HT synthesis. The resultant decrease in 5-HT production may relate to the monoamine hypothesis of major depression. Furthermore, metabolites of the KYN pathway have neurotoxic/neuroprotective activities; 3-hydroxykynurenine and quinolinic acid are neurotoxic, whereas kynurenic acid is neuroprotective. The hippocampal atrophy that appears in chronic depression may be associated with imbalances in neurotoxic/neuroprotective activities. Because proinflammatory cytokines also activate the hypothalamo-pituitary-adrenal (HPA) axis, these imbalances may inhibit the hippocampal negative feedback system. Thus, changes in the TRP metabolism may also relate to the HPA axis-hyperactivity hypothesis of major depression. In this article, we review the changes in TRP metabolism by proinflammatory cytokines under stress, which is assumed to be a risk factor for major depression, and the relationship between physiological risk factors for major depression and proinflammatory cytokines.

**Keywords:** Stress, depression, proinflammatory cytokines, tryptophan, kynurenine, serotonin

### Introduction

Changes in the metabolism of the essential amino acid tryptophan (TRP), play an important role in the brain–endocrine–immune system interaction that is hypothesized to be involved in the pathophysiology of major depression. Two main pathways metabolize TRP: one is the kynurenine (KYN) pathway, which is initiated by the enzyme indoleamine 2,3-dioxygenase (IDO) (Hirata et al. 1974), and the other is the serotonin (5-HT) pathway, initiated by the enzyme

tryptophan 5-monoxygenase (Ichiyama et al. 1970; tryptophan hydroxylase (TPH); Figure 1). Most TRP, i.e., ~99% of dietary intake, is metabolized by the former pathway (Stone and Darlington 2002). Immunological challenges such as proinflammatory cytokines (IL-1, IFN- $\alpha$ , IFN- $\gamma$ , and TNF- $\alpha$ ) induce IDO activity (Stone and Darlington 2002; Konsman et al. 2002; Widner et al. 2002; Moffett and Namboodiri 2003; Myint and Kim 2003; Wichers and Maes 2004). Because proinflammatory cytokines can induce depressive symptoms, cytokine-induced

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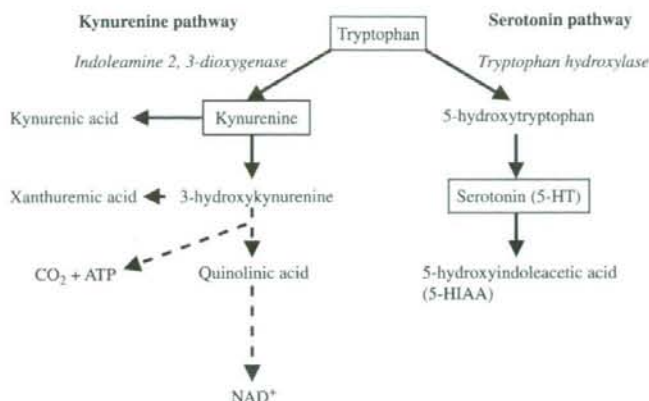


Figure 1. Kynurenine and serotonin pathways of tryptophan metabolism. ATP, Adenosine triphosphate; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; NAD, nicotinamide adenine dinucleotide.

IDO activation may be associated with the etiology of major depression (Konsman et al. 2002; Widner et al. 2002; Wichers and Maes 2004). Activated IDO metabolizes TRP to KYN, and may thereby deprive TPH of its substrate, TRP (Figure 2). Thus, the activated KYN pathway may further inhibit the 5-HT pathway (Konsman et al. 2002; Widner et al. 2002; Myint and Kim 2003; Wichers and Maes 2004). The monoamine hypothesis, one of the main etiological hypotheses of depression, proposes that a decrease in monoamine levels in the central nervous system (CNS) is related to the onset and symptoms of depression (Delgado 2004; Owens 2004). Indeed, lowering 5-HT function by TRP depletion has a mood-lowering effect in the subgroups of recovered

depressed patients, patients with seasonal affective disorder and vulnerable healthy subjects (Van der Does 2001). In the 1970s and 1980s, to clarify the relationship between the amount of KYN metabolites and symptoms of depression, urine levels of xanthurenic acid (XA), which is metabolized from 3-OH KYN (Figure 1), was examined in depressed patients. The excretion of XA after ingestion of TRP was increased in depression, and improvement in XA excretion preceded clinical improvement (Hoes and Sijben 1981). Thus, the induction of IDO activity elicited by proinflammatory cytokines that inhibits 5-HT synthesis may be closely related to the etiological monoamine hypothesis of depression.

The monocyte-T-lymphocyte hypothesis has been proposed to explain the relationship between major depression and immunological activities (Smith 1991; Maes et al. 1995). This theory suggests that the high levels of IL-1 released from activated macrophages directly stimulate the release of corticotrophin-releasing factor (CRF) from the paraventricular nucleus (PVN) in the hypothalamus. Furthermore, IL-6 can also account for hypothalamo-pituitary-adrenal (HPA)-axis abnormalities, due to its direct action on the hypothalamus, again stimulating the release of CRF (Maes et al. 1995). Thus, the HPA-axis hyperactivity hypothesis, which proposes a relationship between HPA-axis hyperactivity and depression (Schule 2007), is closely related to the above-mentioned changes elicited by immunological activation. Both the monoamine and the HPA-axis hyperactivity hypotheses are central to etiological and pathophysiological hypotheses of major depression (Maes et al. 1995; Konsman et al. 2002; Myint and Kim 2003).

Of the metabolites in the KYN pathway (Figure 1), 3-hydroxy kynurenine (3-OH KYN) and quinolinic acid (QUIN) have neurotoxic effects while kynurenic acid (KYNA) has a neuroprotective effect (Myint and

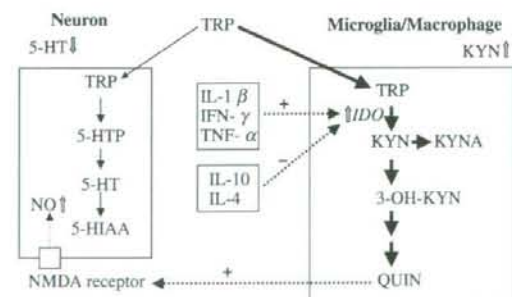


Figure 2. Immunological activation and tryptophan metabolism in the brain. In the macrophages and/or microglia, activated indoleamine 2,3-dioxygenase metabolizes tryptophan. Thus, the kynurenine pathway deprives the serotonin pathway of tryptophan. +, activation; -, inhibition. 3-OH KYN, 3-hydroxykynurenine; 5-HTP, 5-hydroxy tryptophan; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; KYN, kynurenine; KYNA, kynurenic acid; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; QUIN, quinolinic acid; TNF, tumor necrosis factor; TRP, tryptophan.



Kim 2003; Wichers and Maes 2004). Imbalance in the neurodegenerative and neuroprotective activities of KYN pathway metabolites may be linked to hippocampal atrophy in chronic depression, which plausibly relates to HPA-axis hyperactivity in depression. In gerbils, systemic immune activation induces brain IDO and increased brain and CSF QUIN, whereas brain activity of kynurenine aminotransferase (KAT), which converts KYN to KYNA, and the CSF KYNA level are unchanged. Consequently, the QUIN/KYNA ratio in CSF is increased (Saito et al. 1993). D retrovirus infection increases the QUIN/KYNA ratio in the CSF macaques with simian acquired immune deficiency syndrome (SAIDS) (Heyes et al. 1990). In humans, IFN- $\alpha$  therapy revealed that an increase in the serum KYN/KYNA ratio is significantly associated with the severity of depression (Wichers et al. 2005). In patients with major depression, plasma KYNA and the  $1000 \times$  KYNA/KYN ratio are decreased (Myint et al. 2007). Thus, the neurodegeneration process under stress may be more pronounced due to reinforcement by the neurotoxic effect of high QUIN levels (Myint and Kim 2003).

Although the activation of the KYN pathway has been reported in depression elicited by IFN- $\alpha$  therapy (Bonaccorso et al. 2002; Capuron et al. 2002, 2003; Wichers et al. 2005), the therapy is not the typical cause of clinical depression. The onset of clinical depression is more often elicited by social environment and psychological stress (Kendler et al. 1993; Paykel 1994).

In this article, we review the changes in TRP metabolism under physiological conditions and immunological challenge. An interaction between TRP metabolism and neuroendocrine/immune systems is proposed in relation to the etiological hypotheses of depression. To establish and test this hypothesis, it is important to examine changes in TRP metabolism under physiological conditions which are known to be risk factors for depression and to compare them with those elicited by IFN- $\alpha$  therapy.

### Stress and brain tryptophan metabolism

An alteration of TRP metabolism is a good indicator of clinical depression elicited by immunological challenges (Bonaccorso et al. 2002; Capuron et al. 2002, 2003; Wichers et al. 2005) but stress also alters brain TRP metabolism.

#### *Brain tryptophan uptake in response to acute stress and immunological challenge*

Stress as well as immune challenges are known to elevate brain TRP levels. In animals, immobilization stress, food deprivation, exercise, and footshock increase brain TRP levels. Furthermore, immunological challenges such as IL-1 and lipopolysaccharide

(LPS), which are known to activate the HPA axis, increase the brain TRP in mice (Dunn 1988a) and rats (Kabiersch et al. 1988). Because stress-related changes in brain TRP occur even in adrenalectomized rats (Curzon et al. 1972) and mice (Dunn 1988b), they are not dependent on adrenocortical activation. Activation of the sympathetic nervous system is responsible for the increase in brain TRP (Dunn and Welch 1991).

Two related mechanisms increase TRP level in the brain (Figure 3). TRP is the only essential amino acid that binds to albumin (Pardridge 1998). In plasma, most TRP binds to albumin, so free TRP is only a small fraction (McMenamy 1965). One mechanism regulating brain TRP levels is the level of circulating free fatty acid (FFA), under control of the sympathetic nervous system. Stress and physical exercise (Chaouloff 1997) increase plasma FFA level via the sympathetic outflow, and then FFA binds to albumin. Because this binding reduces TRP affinity for albumin (McMenamy 1965), the increase in FFA levels inhibits TRP binding to albumin and increases free TRP levels in the plasma (Curzon et al. 1973; Strüder et al. 1999; Figure 3). The other mechanism regulating brain TRP levels is the competition between TRP and the so-called "large neutral" amino acids (LNAA), which include aromatic amino acids and branched-chain amino acids, at the uptake site of the blood-brain barrier (BBB). The plasma TRP/LNAA ratio, rather than the TRP level, regulates TRP uptake activity at the BBB (Fernstrom and Wurtman 1972; Chaouloff 1997; Strüder et al. 1999; Figure 3). Thus, elevation of the TRP/LNAA ratio increases the brain uptake of TRP, and thereby increases brain TRP level.

Finally, stress may increase the brain TRP level. Our recent data (Miura et al. in press) suggest that 20-min novelty stress in mice, namely, exposure to an environment that the animal has not experienced before, elevates brain TRP levels. Thus, psychological stress possibly increases the brain TRP uptake in humans.

#### *Acute stress and alteration of brain tryptophan metabolism*

Acute psychological as well as physiological stress may alter brain TRP metabolism to activate the brain KYN pathway by induction of IDO.

IDO activity in the brain is present in neurons as well as in microglia and astrocytes (Guillemin et al. 2004; Roy et al. 2005).

In rats, physiological stress such as footshock increases brain TRP, KYN, KYNA and 3-OH KYN levels of rats (Pawlak et al. 2000). Brain TRP levels range from about 10 to 40 nmol/g, whereas those of KYN range from about 400 to 1200 pmol/g. Further, brain levels of KYNA range from about 20 to 40 pmol/g, and those of 3-OH KYN from about 40 to 80 pmol/g

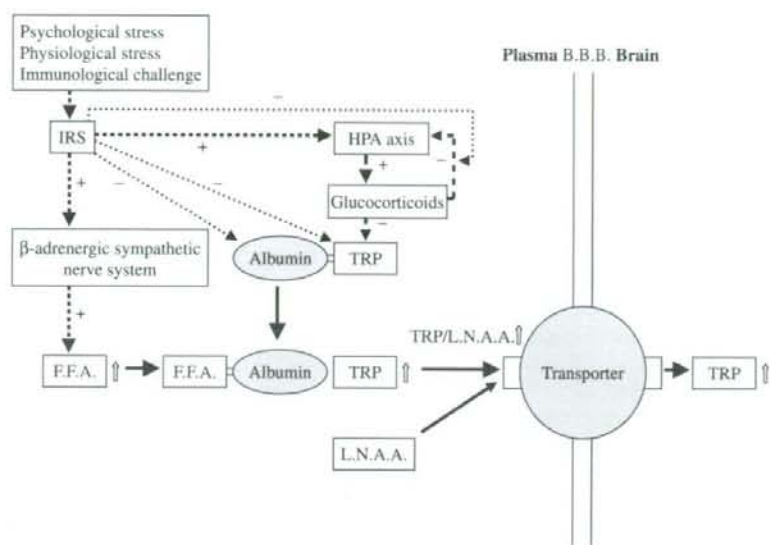


Figure 3. Regulation of plasma level and brain uptake of tryptophan. Stress and immune challenges elevate the brain TRP level. Stimulation of the  $\beta$ -adrenergic system increases FFAs. FFAs bind to albumin, releasing TRP from albumin. Increased plasma TRP elevates the TRP/LNAAs ratio. Thus, the transporter system in the BBB for the uptake into the brain selects TRP rather than LNAAs. Another plasma TRP modulation relates to inflammation. The IRS activates the HPA axis to increase glucocorticoids, consuming TRP and other peripheral amino acids to produce plasma-positive APPs and to reduce plasma negative APPs such as albumin that bind TRP. Thus, HPA axis activity may also play a role in the modulation of plasma TRP levels. APPs, acute-phase proteins; BBB, blood-brain barrier; FFA, free fatty acid; HPA axis, hypothalamo-pituitary-adrenal axis; IRS, inflammatory response system; LNAAs, large neutral amino acid; TRP, tryptophan.

(Pawlak et al. 2000). Thus, brain TRP levels are far greater than those of KYN, KYNA, and 3-OH KYN. As physiological stress is able to increase brain TRP levels via increase of TRP uptake across the BBB, the increase in TRP transfer with stress may compensate for the decrease in TRP elicited by the conversion of TRP to KYN. Thus, brain IDO activity may be effective in physiological stress conditions without apparent immunological challenge. Our recent data (Miura et al. in press) suggest that 20-min novelty stress, i.e., exposure of animals to a condition they have never experienced before, elevated brain KYN levels as well as TRP levels without changing the KYN/TRP ratio. Thus, IDO may play a role in the elevation of the KYN level, accompanied by an increased TRP level elicited by acute psychological stress. Further, novelty stress decreased the 5-HT/TRP ratio. Thus, novelty stress shifted the balance between the KYN and the 5-HT pathways away from 5-HT.

However, influences of chronic and/or repeated stress on brain TRP metabolism, which may relate to the etiology of human depression, should be examined further.

### Stress and proinflammatory cytokines

Exposure to stressors such as psychological stress activates the production of proinflammatory cytokines in humans. Psychological stress, specifically, an

academic examination for medical students, significantly increases blood proinflammatory cytokines (Maes et al. 1998).

The brain-immune system interaction forms a bidirectional communication network, i.e., from the peripheral immune system to the brain and *vice versa* (Black 1994; Maier 2003). Peripherally activated proinflammatory cytokines released from macrophages signal to the brain via both humoral and neural routes. Because proinflammatory cytokines are relatively large and hydrophilic molecules, they are unlikely to cross the BBB by passive diffusion. They pass through the circumventricular organs (CVOs) and choroid plexus, and enter the brain parenchyma. At the CVOs, IL-1 $\beta$  induces, as a second messenger, the formation of prostaglandin E<sub>2</sub>, a small lipophilic molecule that is able to diffuse freely (Maier 2003). Furthermore, in addition to passive diffusion across the BBB, proinflammatory cytokines may enter the brain by active transport mechanisms (Banks et al. 1995; Watkins et al. 1995; Schiepers et al. 2005). Saturable transport mechanisms have already been identified for IL-1 $\beta$ , IL-1 $\alpha$ , IL-6, and TNF- $\alpha$  (Banks et al. 1995). IL-1 $\beta$  also acts on glial cells in the CVOs, meninges, and choroid plexus to stimulate the production of IL-1 $\beta$ . Thus, IL-1 $\beta$  further induces *de novo* IL-1 $\beta$  production by glial cells in a paracrine manner in the brain. This process can propagate the IL-1 $\beta$  signal to distant regions (Maier 2003).



Cytokine signaling to the brain by neural routes involves the vagus nerve. IL-1 $\beta$  released from cells located near vagal afferent terminals can activate nerve impulses. However, the vagus may not be the only nerve that can provide this communication function (Maier 2003).

In addition, the newly synthesized IL-1 $\beta$  in the brain activates both the HPA axis and the sympathetic nervous system in response to peripheral immune stimulation (Maier 2003). Furthermore, cytokines that are produced in the brain enter the peripheral circulation.

An overlap between neurochemical changes elicited by stressors and immune challenges has been frequently noted. Stressors may activate peripheral host-defensive responses, if they elicit bidirectional, immune-brain and brain-immune communication. Stressors such as inescapable shock activate macrophage functions for several days. Novel environmental stress such as exposure to an "open field" increases IL-6 release from macrophages in rats (LeMay et al. 1990). Psychological stress increases IL-1, IL-6, sIL-2R, and cortisol concentrations in human subjects (Schulte et al. 1994). In the brain, acute stress indeed increases the IL-1 $\beta$  content (Minami et al. 1991; Shintani et al. 1995; Nguyen et al. 1998). Moreover, central and peripheral proinflammatory cytokine expression elicited by acute stress is region- and cytokine-specific (O'Connor et al. 2003). Previous exposure to a stressor increases LPS-stimulated IL-1 $\beta$  production (Johnson et al. 2002). Thus, stress may sensitize microglia to respond in a heightened fashion to a subsequent peripheral immune stimulus (Frank et al. 2007).

Furthermore, CRF may play a role in the interaction between a stressor and the production of proinflammatory cytokines (Leonard 2005). As CRF receptors are present on most immune cells such as the resident macrophages of the mouse spleen, human monocytes, macrophages, and T-lymphocytes (Schäfer et al. 1997), stressors may increase proinflammatory cytokines via CRF receptor stimulation (Leonard 2005).

#### Proinflammatory cytokines and tryptophan metabolism

Proinflammatory cytokines induce IDO activity. Subsequent activation of the KYN pathway results in TRP depletion that inhibits cell growth and proliferation, and the 5-HT biosynthesis (Figure 2).

As described above, metabolites in the KYN pathway have a neurotoxic or neuroprotective activity (Figure 1). 3-OH KYN and QUIN have a neurotoxic effect, whereas KYNA has a neuroprotective effect (Myint and Kim 2003; Wichers and Maes 2004). Some of the destructive properties of 3-OH KYN might be caused by its metabolism to 3-hydroxyanthranilic acid, which can undergo auto-oxidation with the formation of superoxide anions (Okuda et al. 1996; Stone and

Darlington 2002; Wichers and Maes 2004). QUIN has the property of being a selective N-methyl-D-aspartate (NMDA) receptor agonist (Stone and Darlington 2002; Myint and Kim 2003; Wichers and Maes 2004). A recent *in vitro* study reported that QUIN reduces antioxidant defenses in the cerebral cortex (Leipnitz et al. 2005). In contrast, KYNA may have neuroprotective properties because it has the properties of a NMDA receptor antagonist and could have agonist properties on nicotinic acetylcholine (nACh) receptors such as the  $\alpha$ 7-nACh receptor (Stone and Darlington 2002; Myint and Kim 2003). The ratio of QUIN/KYNA is increased in the CSF of macaques with AIDS (Heyes et al. 1990). Systemic immune activation in gerbils induces brain IDO, with no change in KAT activity, so the ratio of QUIN/KYNA in CSF increases (Saito et al. 1993). Human microglia as well as macrophages produced KYN and QUIN in cultures activated by IFN- $\gamma$  (Heyes et al. 1996). These findings suggest that immunological challenges including a direct application of IFN- $\gamma$  activate the KYN pathway and QUIN production in the CNS, but do not affect KYNA production.

Thus, the balance of neurotoxic and neuroprotective activities of these metabolites of TRP may play an important role in the pathogenesis of neurodegenerative disease involving depression.

#### Proinflammatory cytokines and the HPA axis

As described above, proinflammatory cytokines activate the HPA axis (Maier 2003; Figure 4). IL-1 is a potent stimulus of CRF synthesis, which supports the activation of the HPA axis (Black 1994; Leonard 2001). The production of prostaglandins (PGs) of the E2 series (PGE2s) at the level of the BBB transmits messages from the cytokines (Konsman et al. 2002). Synthesis of PGE2 is dependent on the induction of cyclooxygenase 2 (COX-2) and PGE synthase expressed in endothelial cells of the cerebral blood vessels and possibly perivascular macrophages. PGE2 activates the CRF neurons of the PVN. These processes activate the HPA axis (Konsman et al. 2002).

Further, the proinflammatory cytokine IFN- $\gamma$  induces IDO expression in hypothalamic and pituitary cells (Tu et al. 2005). The activated IDO in these cells may produce 3-OH KYN and QUIN, which may result in neurodegenerative changes in the HPA axis. Hippocampal atrophy and subsequent inhibition of the negative feedback activity in the HPA axis in chronic depression might be associated with chronic exposure to these neurotoxic products of TRP.

#### Proinflammatory cytokines and brain monoamines

Proinflammatory cytokines act on brain monoamine systems in a cytokine- and region-specific manner in a

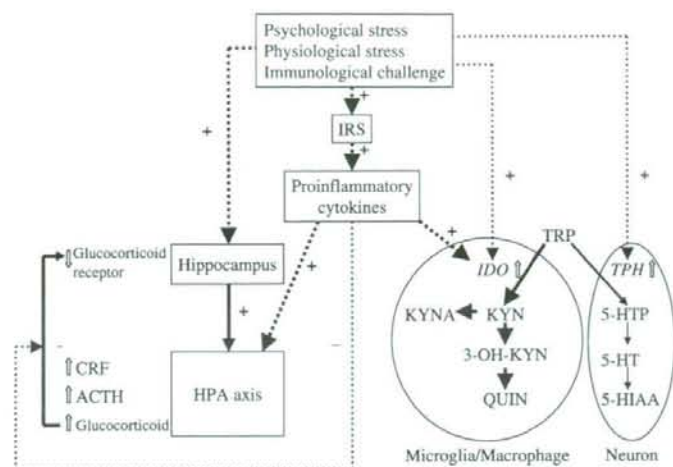


Figure 4. Regulation of brain KYN pathway and HPA axis by stress and immunological challenges. Stress and immune challenges activate the KYN pathway and the HPA axis. Proinflammatory cytokines activate the KYN pathway via induction of IDO, whereas they stimulate the HPA axis. Furthermore, proinflammatory cytokines attenuate the negative feedback control of the HPA axis. ACTH, adrenocorticotropic hormone; CRF, corticotropin releasing factor; 5-HIAA, 5-hydroxyindoleacetic acid; HPA axis, hypothalamo-pituitary-adrenal axis; 5-HT, serotonin; 5-HTP, 5-hydroxy tryptophan; IDO, indoleamine 2,3-dioxygenase; IRS, inflammatory response system; KYN, kynurenine; KYNA, kynurenic acid; 3-OH KYN, 3-hydroxykynurenine; QUIN, quinolinic acid; TRP, tryptophan; TPH, tryptophan hydroxylase.

complex way (Zalcman et al. 1994). As described above, proinflammatory cytokines may reduce the activity of 5-HT pathway by promoting the KYN pathway due to activation of IDO. Although a single i.p.  $\text{INF-}\alpha$  injection has no significant effect on the levels of monoamines and their metabolites or monoamine turnover, repeated i.p. injection significantly decreases both dopamine (DA) and 3,4-dihydroxyphenylacetic acid levels in the mouse brain (Shuto et al. 1997). Acute intracerebroventricular infusion of  $\text{INF-}\alpha$  increases 5-HT turnover in the prefrontal cortex and increases DA turnover in the hippocampus, while pre-treatment with diclofenac, a non-steroidal anti-inflammatory drug prevents these neurochemical responses in rats (De La Garza II et al. 2003). Chronic i.p. administration of  $\text{INF-}\alpha$  to rats reduces DA levels in the prefrontal cortex, decreases 5-HT levels, and increases 5-HT turnover in the amygdala (De La Garza II et al. 2005). In other experiments, although chronic i.p. administration of  $\text{INF-}\alpha$  to rats increases 5-HT turnover in the frontal cortex, hippocampus, amygdala, thalamus, hypothalamus, and brainstem, these treatments do not alter 5-HT transporter mRNA level (Sato et al. 2006). Acute i.p. injection of  $\text{IL-1}\alpha$  to rats increases 5-HT release from the CA1 region of the hippocampus, whereas this treatment decreases norepinephrine (NE) release (Broderick 2002).  $\text{IL-2}$  increases hypothalamic and hippocampal NE utilization and DA turnover in the prefrontal cortex, whereas  $\text{IL-6}$  induces profound elevations of 5-HT and mesocortical DA activity in the hippocampus and prefrontal cortex (Zalcman et al. 1994).

Thus, although the effects of cytokines on brain monoamines are complex, these changes in the activities of brain monoamine systems elicited by proinflammatory cytokines may be related to the etiology and pathophysiology of depression.

#### Proinflammatory cytokines and depression

It is now generally accepted that psychological stress and psychiatric illness can compromise immune functions (Leonard 2001). There is strong evidence to suggest that changes in cytokine levels outside the brain cause changes in cytokine expression and activity in the brain. Conversely, cytokine changes in the brain elicit responses outside the brain (Dantzer et al. 1999). Proinflammatory cytokines induce neuroendocrine and behavioral changes similar to depression in animal models (Dantzer et al. 1999), and immune therapy using proinflammatory cytokines causes depressive symptoms in human subjects (Bonaccorso et al. 2002; Capuron et al. 2002, 2003; Wichers et al. 2005). In addition, changes in the immune system activity, especially the increased levels in proinflammatory cytokines, are found in human depressive patients (Smith 1991; Maes et al. 1995). Thus, depression may now be considered a disorder that is closely related to dysfunction in the immune-endocrine system (Leonard 2001).

Proinflammatory cytokines induce neuroendocrine and behavioral changes in animals that appear similar to those in depressed patients (Dantzer et al. 1999). In terms of neurochemical changes, proinflammatory



cytokines increase the production of CRF, which can escape glucocorticoid negative feedback. In terms of behavioral changes, the anhedonia and helplessness that may be produced by proinflammatory cytokines are considered two essential features of a major depressive episode with melancholia.

An *in vitro* study has shown that the antidepressants clomipramine, sertraline, and trazodon at concentrations of  $10^{-6}$  M (similar to the plasma concentrations during clinical treatment) markedly reduce the proinflammatory cytokine IFN- $\gamma$ , but increase the immune-inhibiting cytokine IL-10 release from human blood (Song 2000). Other studies showed that different antidepressants significantly inhibit proinflammatory cytokine secretion from human monocytes activated by LPS and increase the production of anti-inflammatory cytokines (Xia et al. 1996; Kubera et al. 2000). In contrast, *ex vivo* data collected in depressed patients are much less demonstrative, probably because of the heterogeneity of the patients and their clinical conditions (Haack et al. 1999). However, these actions of antidepressants in *in vitro* studies may help to explain the suppressive effects of antidepressants on proinflammatory cytokine production *in vivo* and on the immune function in depression (Song 2000; Castanon et al. 2002). The existence of 5-HT receptors on human immunocytes suggests a potential mechanism for the immune activity of antidepressants (Maes 2001). 5-HT significantly suppresses the production of IFN- $\gamma$ , whereas the common activity of other antidepressants is to increase IL-10 secretion (Maes 2001). Thus, the suppressive effects of antidepressive drugs on the IFN- $\gamma$ /IL-10 ratio are probably not related to the direct effects of 5-HT. Another possible mechanism of 5-HT revolves around cAMP. 5-HT stimulates 5-HT receptors on immunocytes and increases the intracellular levels of cAMP by a direct activation of adenylyl cyclase. Increased intracellular cAMP levels are considered to play a critical role in the production of IL-10 and IFN- $\gamma$  (Maes 2001). Indeed, tricyclic antidepressants (TCAs) as well as selective serotonin reuptake inhibitors (SSRIs) significantly elevate intracellular cAMP levels in T-lymphocytes and monocytes (Xia et al. 1996). However, the exact mechanisms by which antidepressants exert their effects on proinflammatory cytokine production are still unknown.

#### *Proinflammatory cytokines and the monoamine hypothesis of depression*

As described above, the increase in proinflammatory cytokines in depression and the depressive symptoms following their administration may change brain monoamine system activity. Proinflammatory cytokines have been implicated in the alteration of presynaptic 5-HT as well as NE activities in the

brain regions assumed to be involved in major depression, including the prefrontal cortex, hypothalamus, hippocampus, and amygdala (Zalcman et al. 1994; Broderick 2002; De La Garza II et al. 2003, 2005; Schiepers et al. 2005; Sato et al. 2006). Thus, possible explanations for the association between proinflammatory cytokines and dysfunction of monoaminergic systems in depression have been proposed. Proinflammatory cytokines regulate 5-HT transporter activity (Ramamoorthy et al. 1995; Mössner et al. 1998) and/or the number or sensitivity of postsynaptic 5-HT receptors, including the 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors (Abe et al. 1999). In addition, proinflammatory cytokines stimulate COX-2 activity and thereby increase the synthesis of PGE<sub>2</sub>. Thus, an increase in the plasma and cerebrospinal fluid PGE<sub>2</sub> levels reported in depressed patients may contribute to dysfunction in the release and uptake of brain monoamines by impeding the release of the amines (Leonard 2001).

Thus, activation of the inflammatory response system (IRS) and administration of proinflammatory cytokines may induce depressive symptoms through, among other mechanisms, their modulation of the 5-HT system (Bonaccorso et al. 2000).

#### *Proinflammatory cytokines and HPA axis-hyperactivity hypothesis of depression*

The best-documented changes in biological psychiatry are the increase in the activity of the HPA axis and glucocorticoid resistance in depressed patients (Dantzer et al. 1999). Proinflammatory cytokines might play a causal role in depression mainly because they provide another stimulatory input to the CRF system. The monocyte-T-lymphocyte hypothesis (Smith 1991; Maes et al. 1995) proposes that activated macrophages play a role in the clinical onset and pathophysiology of major depression, and suggests that high levels of IL-1 released from activated macrophages directly stimulate CRF release by the PVN neurons in the hypothalamus. According to this theory, cytokines would increase the reactivity of the HPA axis to external stressors by inducing the expression of vasopressin, another ACTH secretagogue, in parvocellular CRF neurons of the PVN. The chronically increased CRF release can by itself alter the production and action of brain cytokines (Dantzer et al. 1999). Proinflammatory cytokines activate the HPA axis and increase the release of glucocorticoids, which have strong immunosuppressive effects. Thus, the immunosuppression observed in major depression may be due to HPA axis hyperactivity elicited by proinflammatory cytokines and/or stress. The reciprocal relationships between HPA axis activity and the immune system may participate in the pathophysiology of major depression (Maes et al. 1995).



## Depression and tryptophan metabolism

### *HPA axis activity and TRP availability, including plasma TRP level and TRP entry into the brain*

The synthesis of 5-HT in the brain is directly dependent on brain TRP levels. In animals, tryptophan 2,3-dioxygenase (TDO; Hayaishi et al. 1957), another metabolizing enzyme of TRP, is induced by hydrocortisone. Although there is no direct evidence of TDO induction by steroids in humans, this effect may mediate a reduction in TRP availability in response to hypercortisolaemia (Porter et al. 2004; Figure 3). As described above, brain TRP levels are mainly regulated by two mechanisms, competitive binding to plasma albumin with FFA and competition with LNAA at the BBB (Figure 3). Because glucocorticoid treatment induces hepatic and brain tyrosine aminotransferase, which is the major tyrosine-degrading enzyme (Hirota et al. 1985), tyrosine levels may also be altered by glucocorticoid levels (Porter et al. 2004). Further, tyrosine is one of the competing LNAAs at the BBB. Thus, glucocorticoid levels may regulate plasma TRP levels and TRP entry into the brain in a complex manner.

Glucocorticoids are known to attenuate the plasma TRP level and the TRP/LNAA ratio. However, the effects of glucocorticoids on TRP availability in healthy volunteers remain controversial. In healthy volunteers, dexamethasone significantly decreases plasma TRP and tyrosine levels as well as the plasma TRP/LNAA ratio (Maes et al. 1990a). One study reported that dexamethasone does not alter plasma TRP levels (Porter et al. 1999), whereas another study revealed that hydrocortisone significantly decreases plasma TRP (Bhagwagar et al. 2002). In depressive patients, dexamethasone also significantly decreases plasma TRP levels and the plasma TRP/LNAA ratio (Maes et al. 1990a,b). In recovered depression patients, hydrocortisone also suppresses plasma TRP levels (Bhagwagar et al. 2002). Furthermore, patients with major depression and melancholia exhibit significantly lower TRP availability than patients with minor depression (Maes et al. 1990b). Thus, acute administration of glucocorticoids may suppress the availability of TRP (Figure 3).

In addition, cortisol responses to dexamethasone are significantly and negatively associated with TRP availability (Maes et al. 1987, 1990c). However, there is no correlation between TRP/LNAA ratios and basal cortisol output (Maes et al. 1990b,c; Porter et al. 2004). Some other studies suggested that there is no significant difference in the plasma basal TRP levels between controls and depressed subjects (Cowen and Charig 1987; Price et al. 1991), although these studies did not measure LNAA levels or the TRP/LNAA ratio. Thus, the suppression of TRP availability in depression may relate to a dysfunction of the HPA axis

feedback mechanism rather than to the basal level of glucocorticoids (Porter et al. 2004).

The mechanism underlying the attenuation in TRP availability in depression remains unknown. However, the direction of alteration in TRP availability reveals a decrease contrary to the increase in response to acute stress (Figure 3). Acute stress and immunological challenges elevate plasma TRP and the TRP/LNAA ratio, whereas glucocorticoid treatment and depression suppress these measures. These findings suggest that peripheral TRP availability in response to acute stress and immunological challenge causes changes via the  $\beta$ -adrenergic system in the normal physiological condition but via the HPA axis in depression, although the possibility that the TRP changes caused by stress or proinflammatory cytokines have nothing to do with the onset of depression remains to be examined. Furthermore, these changes in depression may be associated with HPA axis feedback mechanism dysfunction rather than a deficiency in the basal level of glucocorticoids (Porter 2004). Stress-induced peripheral immune activation, or IRS, may induce peripheral IDO and/or TDO, and may consume TRP and other peripheral amino acids to produce positive acute-phase proteins (APPs) such as haptoglobin and C-reactive protein (Schiepers et al. 2005). In addition, the inflammation process reduces plasma-negative APPs such as albumin, which bind to TRP (Figure 3). These alterations may modulate the increase in TRP availability in response to acute stress.

Prolonged and/or repeated exposure to stressful life events often precedes the onset of clinical depression. Thus, prolonged adaptation to these stresses may have a consumed ability to protect against new stress on the body and brain, i.e., allostasis load (McEwen 2004). Breakdown of the physiological adjustment system which increases the plasma TRP level, TRP/LNAA ratio, and brain TRP uptake in response to acute stress may result in the suppression of TRP availability.

### *Clinical major depression and tryptophan metabolism*

All the above-described results suggest that proinflammatory cytokines induce depression. Some studies have indicated a correlation between the severity of depressive symptoms and serum TRP decrease and/or KYN increase in depression induced by cytokine therapy (Bonaccorso et al. 2002; Capuron et al. 2002, 2003). In the study of women in pregnancy and delivery, depression and anxiety symptoms in the early puerperium are causally related to an increased catabolism of TRP into KYN (Maes et al. 2002; Kohl et al. 2005). These changes in the plasma TRP and KYN/TRP ratio were significantly related to those in the IL-6 level (Maes et al. 2002).

In patients with major depression, the secretion of proinflammatory cytokines from activated macrophages and the synthesis of APPs from the liver are



increased (Leonard 2001). In studies evaluating plasma TRP and LNAA together with indices of immune functions, patients with depression show decreased plasma TRP levels and TRP/LNAA ratios. Plasma TRP levels negatively correlate to IL-6 production (Maes et al. 1993). Furthermore, depressed subjects have significantly lower serum TRP and albumin levels (Maes et al. 1996). These studies suggest that lower TRP availability to the brain in depression is related to immune responses and lower serum albumin (Maes et al. 1993, 1996).

In recent years, imbalance in the neurodegenerative and neuroprotective activities of KYN pathway metabolites has been revealed as an aspect of depression. IFN- $\alpha$  therapy revealed that a long-term increase of the serum KYN/KYNA ratio, reflecting neurotoxic challenge, is significantly associated with a higher total Montgomery Asberg Depression Rating Scale (MADRS) score (Wichers et al. 2005). Further, plasma KYNA and the neuroprotective ratio,  $1000 \times$  KYNA/KYN, are decreased in patients with major depression (Myint et al. 2007). These studies suggest that the amount and ratio of the neuroprotective metabolite, KYNA, of the KYN pathway are lower in depressive patients. Indeed, patients with major depression who experienced no apparent immunological challenge as well as depressive patients induced by IFN- $\alpha$  showed a decrease in the KYNA level. Thus, a shift in the balance between the neurotoxic and neuroprotective activities of the KYN pathway toward the former may be associated with the etiology of depression.

#### *Effects of antidepressants on the altered tryptophan metabolism*

Although clinical findings support the alteration of TRP metabolism in depression, there is only scant evidence directly indicating the effects of antidepressants in restoring these changes in TRP metabolism. One study reported that paroxetine, an SSRI, did not suppress changes in KYN and neopterin levels elicited by IFN- $\alpha$  therapy in patients with malignant melanoma (Capuron et al. 2003). Further studies evaluating the responses of TRP metabolism to different types of antidepressants in patients with depression as well as in animal models are needed to clarify the role of TRP metabolism in the etiology and/or pathophysiology of depression.

#### **Summary**

In the present review, we explained how changes in TRP metabolism may be involved in the etiology and pathophysiology of depression. Because physiological and/or psychological stress as well as immunological challenges activate the IRS, and then induce proinflammatory cytokine expression both in the periphery

and in the brain, these cytokines may induce and activate peripheral and brain IDO. The activated IDO metabolizes TRP into KYN, and shifts the balance between the KYN and 5-HT pathways of TRP metabolism to the former. Thus, the TRP available for 5-HT synthesis is reduced, and the subsequent 5-HT depletion in the brain may result in depression. The 5-HT shortage caused by IDO activation as well as presynaptic and postsynaptic modulation of the 5-HT system by proinflammatory cytokines may be associated with the monoamine hypothesis of depression. Furthermore, downstream of the activated KYN pathway, the balance of neuroprotective/neurotoxic metabolites may be important; 3-OH KYN and QUIN are neurotoxic, whereas KYNA is neuroprotective. Thus, an imbalance in these metabolites may cause neurodegenerative changes such as hippocampal atrophy in depression. Also, the activation of the HPA axis elicited by proinflammatory cytokines may be closely related to the HPA axis-hyperactivity hypothesis of depression. IRS activation elicited by stress and immunological challenges may thus provide the link between the monoamine and HPA axis-hyperactivity hypotheses of depression via proinflammatory cytokine-induced changes in TRP metabolism. Further accumulation of evidence that directly reveals that IDO induction, a shift in the balance between the KYN and 5-HT systems toward the former, and/or a shift in the balance between the neurotoxic and neuroprotective activities of the KYN pathway toward the former, elicited by physiological and psychological risk factors for depression such as prolonged and repeated exposure to stressful life events would provide support for the importance of the activation of the KYN pathway in the etiology of depression.

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