Changes in brain tryptophan metabolism elicited by ageing, social environment, and psychological stress in mice

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Abstract

The kynurenine (KYN) pathway, which is initiated by indoleamine 2,3-dioxygenase (IDO), is a tryptophan (TRP) metabolic pathway. It shares TRP with the serotonin (5-hydroxytryptamine, 5-HT) pathway. In major depression, activation of the KYN pathway may deplete 5-HT. In the present study we investigated the influence of various risk factors for depression, such as ageing, social isolation and psychological stress, on TRP metabolism. Male ICR mice (postnatal day, PND, 21) were divided into two housing conditions, isolation and group housing, reared for 4 weeks (young adult) or 5 months (adult) and exposed to novelty stress. We measured TRP, KYN and 5-HT contents in the prefrontal cortex, hippocampus, amygdala and dorsal raphe nuclei to investigate the balance between the KYN and 5-HT pathways. Ageing decreased TRP and KYN and increased 5-HT. Thus, ageing shifted the balance to the latter. In the younger group, social isolation decreased TRP and KYN and increased the 5-HT/TRP ratio, whereas novelty stress increased TRP and KYN and decreased the 5-HT/TRP ratio. Thus, social isolation shifted the balance to the latter, whereas novelty stress shifted it to the former. In the older group, these effects were restricted to specific brain regions. Ageing and social isolation counteracted novelty stress effects on TRP metabolism.

Keywords: Tryptophan, kynurenine, serotonin, aging, social isolation, novelty stress

Introduction

Tryptophan (TRP) metabolism has two main pathways: one is the kynurenine (KYN) pathway, which is initiated by the enzyme indoleamine 2,3-dioxygenase (IDO) and the other is the serotonin (5-HT) pathway, which is initiated by the enzyme tryptophan hydroxylase (TPH). These two pathways share TRP with each other, although almost all TRP, i.e. ~99% of dietary intake, is metabolised by the former (Stone and Darlington 2002). Immunological challenges are known to 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). The activated IDO metabolises TRP to KYN, and may deprive TPH of its substrate, TRP. Thus, IDO activation may activate the KYN pathway and lead to the consumption of TRP as the substrate of TPH, resulting in 5-HT depletion (Konsman et al. 2002; Widner et al. 2002; Myint and Kim 2003; Wichers and Maes 2004). The finding that depression is induced by cytokine therapy indicates a correlation between the severity of depressive symptoms and a decrease in serum TRP and/or a KYN increase (Bonaccorso et al. 2002; Capuron et al. 2002, 2003).

To explain the relationship between major depression and immunological activities, a macrophage theory (Smith 1991) indicated that activated macrophages play a role in the clinical onset and pathophysiology of major depression, and suggested that the large amount of IL-1 released from activated...
IDO activation may also influence neurodegenerative and neuroprotective activity. Of the metabolites in the KYN pathway, 3-hydroxy kynurenine (3-OH KYN) and quinolinic acid (QUIN) have neurotoxic effects, whereas kynurenic acid (KYNA) has a neuroprotective effect (Myint and Kim 2003; Wichers and Maes 2004). Thus, the balance between neurodegenerative and neuroprotective effects might relate to hippocampal atrophy in chronically depressed patients.

Patients ordinarily become depressed by adverse life events and/or loss of social support, i.e. psychological and/or environmental factors, especially in older age without severe physical illness or immune therapy (Kendler et al. 1993; Paykel 1994). Hence an investigation of the influence of these factors on TRP metabolism is needed to clarify the above-mentioned hypothesis that a shift in the balance between the KYN and 5-HT pathways to the former explains the etiology and pathophysiology of major depression. An overlap between neurochemical changes elicited by stressors and immune challenges has been frequently noted. Because physiological and/or psychological stress as well as immunological challenges activate the inflammation response system, and then induce proinflammatory cytokines both in the periphery and in the brain, these cytokines may induce and activate peripheral and brain IDO.

However, there is little evidence about the potential influence of ageing, environmental conditions and psychological stress on TRP metabolism. In our recent studies using an animal model, ageing, social environment such as social isolation and acute psychological stress such as exposure to novel environment, did alter brain monoamine turnover in rats (Miura et al. 2002a,b, 2005a) and mice (Miura et al. 2004, 2005b, 2007). Both social isolation and exposure to novel environment are not mild, but strong stressors to mice and rats. Thus, we investigated the relationship between changes in brain TRP metabolism, activities in the KYN and 5-HT pathways, and these three risk factors of major depression using a murine model. We selected four brain regions for study: the prefrontal cortex, because it relates to behavioural motivation; the amygdala, because it relates to emotion; the hippocampus, because it regulates the HPA-axis and hyperactivity of the HPA-axis is closely related to the etiology and pathophysiology of depression; and the dorsal raphe nuclei, because they are the centre of brain 5-HT synthesis. The aim of the present study was 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 modulates the shifting of TRP metabolism.

Materials and methods

Animals

A total of 64 male ICR mice were used in the present experiments. At postnatal day 21 (PND 21), mice that had been housed in groups (7–9 per cage) were divided into two different groups according to housing conditions: i.e. group housing (7–9 per cage; n = 32) or isolation housing (1 per cage; n = 32; Figure 1). After being assigned to one of the two housing conditions, the mice were reared for 4 weeks (young adult group) or 5 months (adult group, Figure 1). The mice were further separated into two groups: in the stress group (n = 32), the mice were exposed to a 20-min novelty stress on the final day; and in the non-stress group (n = 32), the mice were not exposed to the novelty stress (Figure 1). Finally, by combining the above conditions, the mice were divided into 8 groups: young adult, group housing, non-stress; young adult, group housing, stress; young adult, isolation housing, non-stress; young adult, isolation housing, stress; adult, group housing, non-stress; adult, group housing, stress; adult, isolation housing, non-stress; and adult, isolation housing, stress.

The cages used for the group-housing condition were 21 × 31 × 13 cm and the cages used for the isolation-housing condition were 17 × 29 × 13 cm. Cage exchange was performed twice a week for the group-housing group and once a week for the isolation-housing group. Food and water were provided ad libitum. The mice were kept on a 12-h light/dark cycle (lights on 07.00 h, off 19.00 h) and
room temperature was maintained at 21–23°C. All efforts were made to minimise both the number of animals used and the degree of their suffering. All of the experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The study was approved by the ethical committee of Nagoya University Graduate School of Medicine.

Novelty stress test

In the stress group, a 20-min novelty stress session was performed on the final day (i.e. the mice were placed into a transparent plastic box (28 × 35 × 30 cm) that they had not previously experienced). The novelty stress test was performed between 14.00 and 18.00 h in a room that was separate from the holding room, and lit only by a single lamp above the novel cage.

Sample preparation

Mice in the stress group were killed by decapitation immediately after the 20-min stress session, whereas mice in the non-stress group were decapitated without exposure to stress; mice were decapitated under brief ether anaesthesia. The brains were removed and the prefrontal cortex, hippocampus, amygdala and dorsal raphe nuclei were dissected out as quickly as possible on glass plates over ice. The samples were weighed and treated with 1 ml of an ice-cold 0.2 M trichloroacetic acid solution containing 0.2 mM sodium pyrosulphite, 0.01% EDTA-2Na and 0.5 mM sodium 1-octane sulfonate and 5% acetonitrile. The flow rate was 700 μl/min. The concentration of each compound was calculated by comparison with both the internal (ISO) and the external standards.

HPLC determination of brain levels of TRP and KYN

We measured levels of TRP and KYN according to the methods of Widner et al. (1997) and those improved by Laich et al. (2002). A LC-10AD (Shimadzu) HPLC pump was used. For separation, reversed-phase column cartridges LiChroCart 55-4 filled with Purospher STAR Rp-18e (55 mm length, 3 μm grain size) together with a reverse-phase LiChroCART 4-4 precolumn filled with Purospher STAR RP-18e (5 μm grain size, Merck) were used. TRP was detected by RF-535 FD (Shimadzu) at an excitation wavelength of 285 nm and an emission wavelength of 365 nm. KYN and 3-NTYR were detected by a SPD-10A UV-detector (Shimadzu) at a wavelength of 360 nm. The detectors were connected in series to allow simultaneous measurements. The mobile-phase solution consisted of 15 mM L-acetic acid–sodium acetate buffer, pH 4.0, containing 2.7% acetonitrile. The flow rate was 900 μl/min at room temperature.

Statistical analyses

To examine differences in the concentrations of TRP, 5-HT and KYN, and in the ratios of 5-HT/TRP and KYN/TRP, three-way MANOVA (Wilks’s lambda) for ageing, housing condition and novelty stress was conducted on dependent measures in each brain region, followed by the Tukey–Kramer test for ageing. To evaluate the interactions, further analyses were performed. In each age group, i.e. young adult and adult, two-way MANOVA (Wilks’s lambda) for housing condition and novelty stress was conducted on dependent measures in each brain region, followed by the Tukey–Kramer test. P values less than 0.05 were accepted as significant.

Results

The measurements in each brain region are shown in Figure 2 and the corresponding ratios for KYN/TRP and for 5-HT/TRP are shown in Table 1.

Prefrontal cortex

Ageing, housing condition, and novelty stress. The results of three-way MANOVA were as follows: ageing (F(5, 52) = 6.533, P < 0.0001), housing condition (F(5, 52) = 17.115, P < 0.0001) and novelty stress
(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.

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

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 ageing, 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 former. 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.
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. 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 KYNA 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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**References**


