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今回の調査に関するご質問がありましたら、みなさまが所属されている企業の産業医または下記まで、お問い合わせください。

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「職場復帰準備評価シート調査」への同意書（本人）

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私は「職場復帰準備評価シート調査」への参加にあたり、「目的」「計画」「説明同意」「任意性と撤回の自由」「利益および不利益」「個人情報の保護」「調査結果の通知」「調査結果の公表」「費用について」「ご質問など」を理解したうえで、調査への協力に同意いたします。

平成　　年　　月　　日

本人氏名＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

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平成20年度厚生労働科学研究費補助金（こころの健康科学研究事業）
リワークプログラムを中心とするうつ病の早期発見から職場復帰に至る包括的治療に関する研究
分担研究報告書

社会適応評価ツールの有用性の検討
職場復帰に関する生物学的指標の開発

分担研究者　中村　純　　産業医科大学医学部精神医学教室教授（H20年）

研究要旨
本年度は、
1）うつ病患者の社会復帰に対する social adaptation self-evaluation scale（SASS）日本語版の有用性の検討および
2）健常者と休職者している人とを判別するための精神的ストレスの生物学的なマーカー検討を行った。その結果、ベックうつ病評価尺度（BDI）よりもSASSの方が職場復帰の評価尺度として優れており、また血中BDNF，MHPG濃度が現在受けている職場ストレスの強さを反映している可能性が示唆された。

A－1研究目的（研究1）
うつ病患者が改善した場合に求められるのは社会復帰であり、うつ病の改善と復職はかならずしも一致しない。したがって、本年度は、
1）うつ病患者の社会復帰に対する social adaptation self-evaluation scale（SASS）日本語版の有用性の検討および
2）健常者と休職者している人とを判別するための精神的ストレスの生物学的なマーカー検討することを目的とした。具体的には、自記式SASS日本語版の有用性を従来から利用されている自記式うつ病評価尺度の一つであるBDIとを比較検討した。

B－1対象と方法
うつ病の外来患者のうち発病前に就労していた100例（男性：54名、女性：46名、平均年齢43±11歳）を対象にした。対象者を調査時点で就労している社会復帰群（45名）と非社会復帰群（55名）に分け、両群のSASSおよびBDI得点を比較した。さらに各尺度のROC曲線（receiver operating characteristic curve）を作成し、両者の精度を検討した。本研究は産業医科大学倫理委員会の承認を受けており、対象者からは文書による同意を得た。

C－1結果
社会復帰群では非社会復帰群と比較してSASS得点が有意に高く（社会復帰群；35.5±6.9、非社会復帰群23.7±6.4，$P<0.001$）、BDI得点が有意に低かった（社会復帰群：12.5±10.7、非社会復帰群：26.8±11.8，$P<0.001$）。以上の結果からSASS，BDI共に社会復帰の指標となることが示唆された。さらにSASSとBDIの曲面下面積を比較したところ、SASSの方がBDIよりも曲面下面積が大きかった（SASS；0.890，BDI；0.815）。

D－1結論
今回の結果よりBDIよりもSASSの方がより社会復帰の評価尺度として精度が優れていると考えられた。

A－2研究目的（研究2）
血中ＢＤＮＦ（脳由来栄養因子）、カテコールアミン、サイトカイン濃度は、うつ病、自殺、

不安状態だけでなく、うつに関連した人格特性にも関与するというエビデンスが増加しつつある。職場での精神的ストレスがうつ病や不安障害を引き起こすことはよく知られている。そこで本年度は、身体疾患・精神障害に罹患していない健康な勤労者に対して職場ストレスが血中BDNF、カテコールアミン代謝産物（HVA, MHPG)、サイトカイン（IL-6, TNF α）濃度に影響を与えるかを検討した。

B－2 対象と方法

健康な医療従事者（106名、男性：42名、女性64名、平均年齢36±12歳）でStress and Arousal Check List（SACL）の中のストレス項目だけを抽出したs-SACLを施行した。さらに早朝8時に採血と行い、血漿中HVA, MHPG濃度はHPLC-ECD法、血清中BDNF, IL-6およびTNF αはsandwich ELISA法で測定した。本研究は産業医科大学倫理委員会の承認を受けており、対象者からは文書による同意を得た。

C－2 結果

s-SACL得点と血清BDNF濃度の間には負（rho＝−0.211, p＝0.002)、血漿MHPG濃度とは正（rho＝0.416, p＝0.01）の相関が認められた。血漿HVA, IL-6およびTNF αとs-SACL得点には相関がなかった。

D－2 結論

本研究の結果から健康な健常者においても職場での精神的ストレスは血清BDNF濃度を下げ、血漿MHPG濃度を上げる可能性が示された。すなわち、精神的ストレスがノルアドレナリン神経の過活動と関連し、脳内BDNF産生を減少させる可能性があることが示唆された。また、本研究から、血中BDNF, MHPG濃度が職場ストレスの生物学的指標になりうる可能性も考えられる。

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リワークプログラムを中心とする
うつ病の早期発見から復職支援に至る包括的治療に関する研究

分担研究報告書

抗うつ薬の作業能力への影響の検討

分担研究者　尾崎紀夫　名古屋大学大学院医学系研究科精神医学分野

研究要旨：抗うつ薬はうつ病に伴う認知機能障害の回復し、うつ病患者の社会復帰を促しているが、一方で抗うつ薬が回復した認知機能に影響することも報告されている。現代の車社会においては、自動車運転なしでの日常生活や就労業務は困難な状況にある。運転業務を含めて社会復帰を目指した、うつ病患者に対する適切な抗うつ薬薬物療法についてはさらに詳細に検討する余地がある。そこで、本研究においては、健常者を対象に模擬運転装置を用いて、向精神薬を服用した上で、経時的に運転作業課題を施行し、その課題成績と薬物血中濃度の推移を確認する。本年度の結果、1）に関しては、抗うつ薬血中濃度と運転技能・認知機能との関連として，アミトリプチリンが濃度依存性に車線維持運転技能を障害することが確認された。これらの結果から，抗うつ薬の選択によっては，運転を含めた日常業務遂行など、うつ病患者の社会復帰を妨げ，労働災害に結びつく危険性があることが示唆された。また、治療薬物モニタリングにより自動車運転の困難さを予測できる可能性も示唆された。なお抗うつ薬であるパロキセチン血中濃度との関連は現在解析中であり，次年度にはその結果を報告できる予定である。

研究協力者
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A.　研究目的

薬物反応性には個人差が存在する。治療薬物モニタリングを行うことで、用量調節や有効性・安全性を高めることが可能となる。抗うつ薬についても、三環系抗うつ薬では、脱落率や中枢神経系・心血管系毒性との関連から治療薬物モニタリングが推奨されてきた。各種抗うつ薬は、その治療効果は同等であり、うつ病に伴う認知機能障害を回復し、社会復帰を促しているが、一方で、三環系抗うつ薬服用により交通事故発生率が2倍に増加することが疫学的研究によって報告されており、また三環系抗うつ薬が認知機能及び運動機能の障害をもたらし、運転技能にも悪影響を及ぼすことが繰り返し確認されている。血中濃度の個人差によって、運転技能が様々に影響されると考えられるが、薬物血中濃度と運転技能・認知機能との関連は十分に検討されていない。本研究の目的は、個人の薬物動態の差異が運転技能及び認知機能に与える影響を検討し、うつ病患者の社会復帰を目指した、適切な抗うつ薬薬物療法を明確化することである。

B．　研究方法

1）向精神薬血中濃度と運転技能・認知機能の関連研究

被験者として，運転免許を有し、日常的に運転を行う健常男性17名が参加し，問診や精神科診断面接（SCID）により身体疾患や精神障害を有さないことを確認した。被験者の平均年齢は35.8±3.3歳（±に続く数値は標準偏差。以下同様）であり，年齢幅は30～42歳であった。

向精神薬の影響を確認する為に，臨床的に汎用されている，パロキセチン（SSRI）10mg，アミトリプチリン（三環系抗うつ薬）25mg及びプラセボを用いた二重盲検，クロスオーバー試験法（Wash Out期間は1週間以上）を行った。認知機能に与える影響を確認する為に，薬物服用前，服用1時間後，服用4時間後で模擬運転装置による運転業務負荷試験と認知機能試験を行い、各測定時点で主観的な眠気(Stanford眠気尺度）を，試験終了後には副作用をそれぞれ質問紙により確認した。運転業務負荷試験としては，追従走行課題（先行車との車間距離をどれだけ維持できるか），車線維持課題（横方向での揺れの程度），飛び出し課題（ブレーキ反応時間）の3課題を，また認知機能試験としてはCPT（持続的注意），WCST（遂行機能），N-Back test（ワーキングメモリ）の3課題を行った。検査前には各被験者に操作方法を十分に教示した上で試験を行った。試験開始前と追従走行課題施行前後に採血し、速やかに遠心分離による血漿分離後に凍結保存とし、ＨＰＬＣ法により薬物血中濃度を測定した。

倫理的配慮

本研究は名古屋大学倫理審査委員会の承認を得ており、対象者には本研究に関して十分な説明を行い、文書にて同意を取得した。

C．　研究結果

1）向精神薬血中濃度と運転技能・認知機能の関連研究

アミトリプチリン血中濃度は、15.3±6.4ng/ml（濃度幅：8.5-32.9ng/ml）であった。アミトリプチリン血中濃度と運転業務負荷試験および認知機能試験の成績について、相関分析を行ったところ、アミトリプチリン血中濃度と車線維持課題において、有意な正の相関（r＝0.543、P＝0.045、Spearman rank-order correlation）を認めた。他の運転業務負荷試験である追従走行課題および飛び出し課題については、有意な相関を認めなかった（それぞれr＝-0.11、P＝0.673、Spearman順位相関、r＝-0.163、P＝0.532、Pearson積率相関）。認知機能試験の各課題については、CPT(r＝0.209、P＝0.42、Pearson積率相関)、WCSTのカテゴリー達成度(r＝-0.07、P＝0.789、Spearman順位相関)、WCSTの保続性の誤り(r＝0.048、P＝0.855、Spearman順位相関)、WCSTのセットの維持困難（r＝0.132、P＝0.614、Spearman順位相関)、N-back testの2back task（r＝0.26、P＝0.37、Spearman順位相関）と、いずれも有意な相関は認めなかった。主観的な眠気を評価するStanford眠気尺度についても、有意な相関を認めなかった（r＝0.035、P＝0.893、Spearman順位相関)。

なお、アミトリプチリンが運転業務負荷試験、認知機能試験、主観的眠気に及ぼす影響については、内服4時間後において、車線維持課題(P＝0.003)、追従走行課題（P＝0.006)、CPT（P＝0.035)、主観的眠気（P＝0.0002）でそれぞれ有意に障害した。

D．考察と結論

アミトリプチリンは濃度依存性に車線維持課題の成績（車の横揺れ）を有意に障害し、この車の横揺れの増加が交通事故の増加につながると考えられた。その他の運転技能（追従走行課題、飛び出し課題)、認知機能（持続的注意、

遂行機能、ワーキングメモリ)、主観的眠気については、血中濃度との相関は認められなかったが、これらの指標については、個人の薬物動態的差異よりも、個人の薬力学的差異が関与していると考えられた。うつ病患者においても認知機能障害が認められると報告されているが、抗うつ薬がうつ病症状を改善したとしても、アミトリプチリンを含めた三環系抗うつ薬では回復した患者の運転技能・認知機能にも影響を及ぼすことが示唆された。また、アミトリプチリンの治療薬物モニタリングが、運転の困難さを予測する有用性も示唆した。これらの結果から，抗うつ薬の選択によっては，運転を含めた日常業務の遂行など、うつ病患者の社会復帰を妨げ、労働災害にも結びつく危険性があることが示唆された。なお、パロキセチン血中濃度と運転技能・認知機能の関連については次年度に報告したい。今後、抗不安薬であるジアゼパム及びタンドスピロンを用いて，運転技能及び認知機能に与える影響、また薬物血中濃度との関連を調査する予定である。

G．　研究発表

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H．　知的財産権の出願。登録状況

なし

別紙 4

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yoshida K, Higuchi H, Takahashi H, Kamata M, Sato K, Inoue K, Suzuki T, Itoh K, Ozaki N	Influence of the tyrosine hydroxylase val 81met polymorphism and catechol-O-methyltransferase val158 met polymorphism on the antidepressant effect of milnacipran.	Hum Psychopharmacol	23 (2)	121-8	2008
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Influence of the tyrosine hydroxylase val81met polymorphism and catechol-*O*-methyltransferase val158met polymorphism on the antidepressant effect of milnacipran

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Objective Genetic polymorphisms of the noradrenergic pathway can be factors to predict the effect of antidepressants when their pharmacological mechanisms of action include the noradrenergic system. The purpose of the present study was to determine whether the tyrosine hydroxylase (TH) val81met and catechol-*O*-methyltransferase (COMT) val158met polymorphisms are associated with the antidepressant effect of milnacipran, a serotonin/noradrenaline reuptake inhibitor.
Method Eighty-one Japanese patients with major depressive disorder were treated with milnacipran for 6 weeks. Severity of depression was assessed with the Montgomery and Åsberg Depression Rating Scale (MADRS). Assessments were carried out at baseline and at 1, 2, 4 and 6 weeks of treatment. The method of polymerase chain reaction was used to determine allelic variants.
Results The met/met genotype of the COMT val158met polymorphism was associated with a significantly faster therapeutic effect of milnacipran in the MADRS score during this study. No influence of the TH val81met polymorphism on the antidepressant effect of milnacipran was detected.
Conclusion These results suggest that the COMT val158met polymorphism in part determines the antidepressant effect of milnacipran. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS — catechol-*O*-methyltransferase; major depressive disorder; milnacipran; polymorphism; tyrosine hydroxylase

INTRODUCTION

Individual genetic differences of monoaminergic pathways can have an impact on the effect of antidepressant agents, though the exact mechanism of their action is still unclear. Several lines of evidence have suggested the relationship between genetic polymorphisms of the serotonergic pathway, especially those of the 5-hydroxytriptamine transporter (5-HTT), and the antidepressant effect of selective serotonin reuptake inhibitors (SSRIs) (Binder and Holsboer, 2006).

Genetic polymorphisms of the noradrenergic pathway as well as serotonergic pathway could also affect the effect of antidepressants, especially when their pharmacological mechanisms of action include the noradrenergic system. Tyrosine hydroxylase (TH) is

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the initial and rate-limiting enzyme in the biosynthesis of catecholamine neurotransmitters including noradrenaline. The TH val81met polymorphism in exon 2 (Ludecke and Bartholome, 1995) is located in the amino-terminal regulatory domain of the tetrameric enzyme. The regulatory region is reported to have an inhibiting effect on the enzymatic function (Kumer and Vrana, 1996). Catechol-*O*-methyltransferase (COMT) is an important enzyme involved in degradation of catecholamine neurotransmitters including noradrenaline. The COMT val158met polymorphism located in exon 4 (Lotta *et al.*, 1995) was reported to be associated with variation in COMT enzyme activity (Lachman *et al.*, 1996).

No pharmacogenetic study addressed the relationship between the TH val81met polymorphism and antidepressant response. Only two studies investigated the relationship between the COMT val158met polymorphism and antidepressant response to SSRIs and mirtazapine. One reported its no overall effect on the antidepressant response to SSRIs (Arias *et al.*, 2006), and the other reported its significant effect on the antidepressant response to mirtazapine but not paroxetine (Szegedi *et al.*, 2005).

So far, there has been no study investigating the relationship between the TH val81met polymorphism, the COMT val158met polymorphism and the antidepressant response to serotonin noradrenaline reuptake inhibitors (SNRIs), although noradrenergic genetic factors could be one of the most plausible candidates for pharmacogenetic analysis of SNRIs. The class of SNRIs now comprises of three medications: venlafaxine, duloxetine and milnacipran. Among SNRIs, venlafaxine has a high affinity for the 5-HTT but not the noradrenaline transporter. Duloxetine has a more balanced affinity but is still more selective for the 5-HTT. Milnacipran is the most balanced and may even be slightly more noradrenergic than serotonergic (Stahl *et al.*, 2005). Thus, the authors investigated whether the above two noradrenergic polymorphisms affect the antidepressant effect of milnacipran.

SUBJECTS AND METHODS

Subjects

For the present study, one subject treated with milnacipran was added to those in our previous study (Yoshida *et al.*, 2007). Detailed inclusion criteria have been described previously (Yoshida *et al.*, 2007). In brief, the subjects were Japanese patients who fulfilled DSM-IV criteria for a diagnosis of major depressive disorder and whose scores on the Montgomery Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979) were 21 or higher. Patients with other axis I and II disorders determined by clinical interview and those with severe nonpsychiatric medical disorders were excluded. The patients were 25–69 years of age (mean age (±SD) = 51.1 ± 12.3) and had been free of psychotropic drugs at least 14 days before entry into the study. After complete description of the study to the subjects, written informed consent was obtained. This study was approved by the Ethical Committee of Akita University School of Medicine and Nagoya University Graduate School of Medicine. The clinical characteristics of the patients are shown in Table 1. There was no significant difference between responders and nonresponders in regard to sex, age, number of previous episodes and presence of melancholia.

Milnacipran treatment

Milnacipran was administered twice daily (the same dose after dinner and at bedtime) for 6 weeks. The initial total daily dose was 50 mg/day, and after a week it was increased to 100 mg/day. Patients with insomnia were prescribed 0.25 or 0.5 mg of brotizolam, a benzodiazepine sedative hypnotic, at bedtime. No other psychotropic drugs were permitted during the study. Of 98 enrolled patients, 10 did not complete the study: five patients because of side effects, one patient because of severe insomnia and four patients without explanation. Of the 88 patients who completed the 6-week study, seven patients were excluded from the

Table 1. Clinical characteristics of the patients (responders and nonresponders)

	Responders ($n = 51$)	Nonresponders ($n = 30$)		p
Sex (male/female)	20/31	9/21	$\chi^2 = 0.70$	0.40[a]
Age (year) (±SD)	50.7 ± 12.4	51.8 ± 12.2	$t = -0.41$	0.68[b]
No. of previous episodes (±SD)	0.47 ± 1.3	0.23 ± 0.6	$t = 0.97$	0.34[b]
Melancholia (+/−)	16/35	9/21	$\chi^2 = 0.017$	0.90[a]

[a]Analysis performed with the use of the χ^2 test.
[b]Analysis performed with the use of the unpaired t-test.

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current analysis because plasma samples revealed very low milnacipran concentrations, indicative of poor compliance. Patients who completed the study included 52 women and 29 men, 50 outpatients and 31 inpatients.

Data collection

Depression symptom severity was assessed with the use of the MADRS. Assessments were conducted at baseline and at 1, 2, 4 and 6 weeks after initiation of antidepressant treatment. A single rater conducted each of the ratings for each patient. A clinical response was defined as a 50% or greater decrease in the baseline MADRS score. Clinical remission was defined as a final MADRS score less than 10 (Hawley *et al.*, 2002). Collection of blood samples was performed 12 h after drug administration at bedtime, 4 weeks after initiation of antidepressant treatment.

Genotyping

The TH val81met polymorphism was determined by the method of Sharma *et al.* (1998). The COMT val158met polymorphism was determined by the method of Lachman *et al.* (1996).

Quantification of plasma milnacipran concentration

Plasma concentrations of milnacipran were measured with high performance liquid chromatography (HPLC). Details of the method have been described previously (Higuchi *et al.*, 2003). Genotyping and measurement of plasma concentrations were performed by laboratory personnel blind to the identity and clinical antidepressant effect of the patients. Moreover, clinicians were unaware of the genotyping results and the plasma milnacipran concentrations of each patient.

Statistical analysis

Differences in patient characteristics were analysed with the use of the unpaired *t*-test or Chi-square test where appropriate. Differences in the MADRS scores during this study were examined with the use of two-way repeated-measures analysis of variance (ANOVA), with genotype and time as factors. Additional repeated-measures analysis of covariance (ANCOVA) was performed if necessary. When significant interaction between factors was observed, contrasts were used to enable comparisons between each two of the three genotype groups. Differences in the MADRS scores at each evaluation point were examined with the one-way factorial ANOVA followed by the Fisher's PLSD test. Genotype deviation from the Hardy–Weinberg equilibrium was evaluated by the Chi-square test. Genotype distribution and allele frequencies were analysed with the use of the Chi-square test. Plasma concentrations of milnacipran were analysed with the use of one-way factorial ANOVA in each genotype group; an unpaired *t*-test was then used to analyse differences between groups who were or were not responsive to milnacipran. Statistical analysis was performed using StatView version 5.0 (SAS Institute, Inc., Cary, NC) and SuperANOVA version 1.11 (Abacus Concepts, Inc., Berkeley, CA). Power analysis was performed with the use of G·Power (Buchner *et al.*, 1996). All tests were two-tailed; alpha was set at 0.05.

RESULTS

TH val81met polymorphism

The observed genotype frequencies of the TH val81met polymorphism were within the distribution expected according to the Hardy–Weinberg equilibrium. Figure 1 shows the MADRS scores over time in relation to the TH val81met polymorphism. Two-way repeated-measures ANOVA including all three genotype groups indicated no significant genotype × time interaction ($F = 0.99$, df = 8, $p = 0.44$). Plasma concentrations of milnacipran were not significantly different among each genotype group (val/val: 96.1 ± 32.6 (±SD), val/met: 86.2 ± 30.4, met/met: 92.2 ± 47.9, $F = 0.35$, df = 2, $p = 0.71$). No significant differences in the genotype

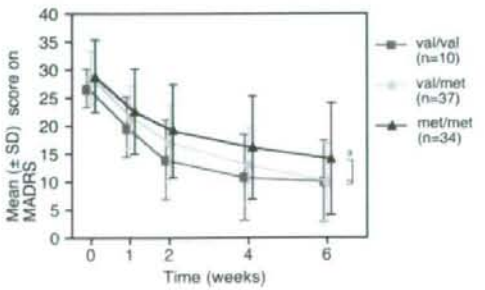

Figure 1. MADRS scores during 6 weeks of the treatment in three TH val81met genotype groups. (Each point represents the mean score ± SD. Differences in the MADRS scores during this study were examined with the use of repeated-measures ANOVA.) "There was no significant genotype × time interaction among all three genotype groups ($F = 0.99$, df = 8, $p = 0.44$)

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Table 2. Genotype distribution and allele frequencies in responders/nonresponders and remitters/nonremitters in the TH val81met polymorphism[a]

	Genotype distribution[b,c]			Allele frequency[d,e]	
	val/val	val/met	met/met	val	met
Responder	7 (13.7%)	27 (52.9%)	17 (33.3%)	41 (40.2%)	61 (59.8%)
Nonresponder	3 (10.0%)	10 (33.3%)	17 (56.7%)	16 (26.7%)	44 (73.3%)
Remitter	5 (11.4%)	23 (52.3%)	16 (36.3%)	33 (37.5%)	55 (62.5%)
Nonremitter	5 (13.5%)	14 (37.8%)	18 (48.7%)	24 (32.4%)	50 (67.6%)

[a]Analysis performed with the use of the χ^2 test.
[b]No significant difference between responders and nonresponders ($\chi^2 = 4.25$, df = 2, $p = 0.12$).
[c]No significant difference between remitters and nonremitters ($\chi^2 = 1.72$, df = 2, $p = 0.42$).
[d]No significant difference between responders and nonresponders ($\chi^2 = 3.03$, df = 1, $p = 0.08$).
[e]No significant difference between remitters and nonremitters ($\chi^2 = 0.45$, df = 1, $p = 0.50$).

distribution ($\chi^2 = 4.25$, df = 2, $p = 0.12$) and allele frequencies ($\chi^2 = 3.03$, df = 1, $p = 0.08$) were noted between responders and nonresponders. When remitters and nonremitters were compared, there was also no significant difference in the genotype distribution ($\chi^2 = 1.72$, df = 2, $p = 0.42$) and allele frequencies ($\chi^2 = 0.45$, df = 1, $p = 0.50$) (Table 2).

COMT val158met polymorphism

The observed genotype frequencies of the COMT val158met polymorphism were within the distribution expected according to the Hardy–Weinberg equilibrium. Figure 2 shows the MADRS scores over time in relation to the COMT val158met polymorphism. Two-way repeated-measures ANOVA including all three genotype groups indicated a significant genotype × time interaction ($F = 2.00$, df = 8, $p = 0.046$). Contrast analysis indicated a significant genotype × time interaction between the val/met and met/met genotype groups ($F = 3.31$, df = 4, $p = 0.011$). The MADRS score of the val/met genotype group was significantly lower than that of the met/met genotype group at the 0 week ($p = 0.0098$). Contrast analysis indicated a significant genotype × time interaction between the val/val and met/met groups ($F = 3.19$, df = 4, $p = 0.011$). The MADRS score of the val/val genotype group was significantly lower than that of the met/met group at the 0 week ($p = 0.013$). Contrast analysis indicated no significant genotype × time interaction between the val/val and val/met genotype groups ($F = 0.49$, df = 4, $p = 0.74$). There was no significant difference in the MADRS score at any evaluation point between the val/val and val/met genotype groups. To determine whether the initial difference of the MADRS scores affect the subsequent scores, a repeated measures ANCOVA was performed with the initial MADRS score as a covariate. This analysis revealed no significant time × the initial MADRS score interaction ($F = 0.46$, df = 3, $p = 0.71$), indicating that the initial MADRS score was not a significant covariate.

To determine which aspects of depressive symptoms contributed to overall differences over time of the MADRS scores, the results of factor analyses of depression symptomatology using MADRS (Parker *et al.*, 2003; Suzuki *et al.*, 2005) were applied to the present results. Suzuki *et al.* (2005) identified three factors labelled dysphoria, retardation and vegetative symptoms. Figure 3 shows the dysphoria scores over time in relation to the COMT val158met polymorph-

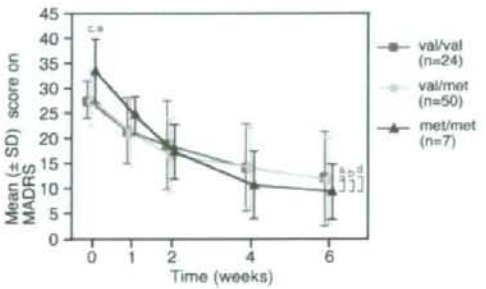

Figure 2. MADRS scores during 6 weeks of the treatment in three COMT val158met genotype groups. (Each point represents the mean score ± SD. Differences in the MADRS scores during this study were examined with the use of repeated-measures ANOVA. Differences in the MADRS scores at each evaluation point were examined with the use of one-way factorial ANOVA followed by Fisher's PLSD test.) [a]Significant genotype × time interaction among all three genotype groups ($F = 2.00$, df = 8, $p = 0.046$). [b]Significant genotype × time interaction between the val/met and met/met groups ($F = 3.31$, df = 4, $p = 0.011$). [c]Significant difference at the 0 week between the val/met and met/met groups ($p = 0.0098$). [d]Significant genotype × time interaction between the val/val and met/met groups ($F = 3.19$, df = 4, $p = 0.011$). [e]Significant difference at the 0 week between the val/val and met/met groups ($p = 0.013$)

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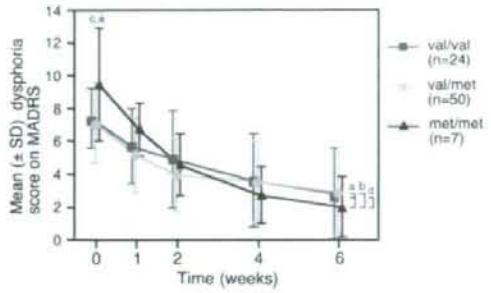

Figure 3. MADRS dysphoria scores during 6 weeks of the treatment in three COMT val158met genotype groups. (Each point represents the mean score ± SD. Differences in the MADRS dysphoria scores during this study were examined with the use of repeated-measures ANOVA. Differences in the MADRS dysphoria scores at each evaluation point were examined with the use of one-way factorial ANOVA followed by Fisher's PLSD test.) [a]Significant genotype × time interaction among all three genotype groups ($F = 2.68$, df = 8, $p = 0.0074$). [b]Significant genotype × time interaction between the val/met and met/met groups ($F = 4.43$, df = 4, $p = 0.0017$). [c]Significant difference at the 0 week between the val/met and met/met groups ($p = 0.016$). [d]Significant genotype × time interaction between the val/val and met/met groups ($F = 3.23$, df = 4, $p = 0.013$). [e]Significant difference at the 0 week between the val/val and met/met groups ($p = 0.049$)

ism. Two-way repeated-measures ANOVA for the dysphoria scores including all three genotype groups indicated a significant genotype × time interaction ($F = 2.68$, df = 8, $p = 0.0074$). As in the case of overall results, contrast analysis indicated a significant genotype × time interaction between the val/met and met/met genotype groups ($F = 4.43$, df = 4, $p = 0.0017$), and between the val/val and met/met genotype groups ($F = 3.23$, df = 4, $p = 0.013$). Contrast analysis indicated no significant genotype × time interaction between the val/val and val/met genotype groups ($F = 1.10$, df = 4, $p = 0.36$). Two-way repeated-measures ANOVA for the scores of retardation and vegetative symptoms did not indicate significant genotype × time interactions (data not shown). Parker *et al.* (2003) identified three factors labelled dysphoric apathy/retardation, psychic anxiety and vegetative symptoms. Two-way repeated-measures ANOVA for the scores of psychic anxiety including all three genotype groups indicated a significant genotype × time interaction ($F = 3.24$, df = 8, $p = 0.0015$). As in the case of overall results and those based on the factor analyses by Suzuki *et al.* (2005), contrast analysis indicated a significant genotype × time interaction between the val/met and met/met genotype groups ($F = 5.97$, df = 4, $p = 0.0001$), and between the val/val and met/met genotype groups ($F = 4.47$, df = 4, $p = 0.016$). Contrast analysis indicated no significant genotype × time interaction between the val/val and val/met genotype groups ($F = 0.63$, df = 4, $p = 0.64$). Two-way repeated-measures ANOVA for the scores of dysphoric apathy/retardation and vegetative symptoms did not indicate significant genotype × time interactions (data not shown).

Plasma concentrations of milnacipran were not significantly different among each genotype group (val/val: 82.7 ± 21.6 (±SD), val/met: 94.7 ± 44.9, met/met: 81.1 ± 34.7, $F = 0.97$, df = 2, $p = 0.38$). No significant differences in the genotype distribution ($\chi^2 = 1.79$, df = 2, $p = 0.41$) and allele frequencies ($\chi^2 = 0.81$, df = 1, $p = 0.37$) were noted between responders and nonresponders. When remitters and nonremitters were compared, there was also no significant difference in the genotype distribution ($\chi^2 = 0.93$, df = 2, $p = 0.63$) and allele frequencies ($\chi^2 = 0.16$, df = 1, $p = 0.69$) (Table 3).

Power

This study had a power of 0.12 to detect a small effect, 0.67 to detect a medium effect and 0.99 to detect a

Table 3. Genotype distribution and allele frequencies in responders/nonresponders and remitters/nonremitters in the COMT val158met polymorphism[a]

	Genotype distribution[b,c]			Allele frequency[d,e]	
	val/val	val/met	met/met	val	met
Responder	14 (27.5%)	31 (60.8%)	6 (11.8%)	59 (57.8%)	43 (42.2%)
Nonresponder	10 (33.3%)	19 (63.3%)	1 (3.3%)	39 (65.0%)	21 (35.0%)
Remitter	13 (29.5%)	26 (59.1%)	5 (11.4%)	52 (59.1%)	36 (40.9%)
Nonremitter	11 (29.7%)	24 (64.9%)	2 (5.4%)	46 (62.2%)	28 (37.8%)

[a]Analysis performed with the use of the χ^2 test.
[b]No significant difference between responders and nonresponders ($\chi^2 = 1.79$, df = 2, $p = 0.41$).
[c]No significant difference between remitters and nonremitters ($\chi^2 = 0.93$, df = 2, $p = 0.63$).
[d]No significant difference between responders and nonresponders ($\chi^2 = 0.81$, df = 1, $p = 0.37$).
[e]No significant difference between remitters and nonremitters ($\chi^2 = 0.16$, df = 1, $p = 0.69$).

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large effect in the genotype distribution ($n = 81$). For the allele frequency analysis ($n = 162$), this study had a power of 0.25 to detect a small effect, 0.97 to detect a medium effect and 0.99 to detect a large effect. In the power analysis, effect size conventions were determined according to the method of Buchner *et al.* (1996) as follows: small effect size = 0.10, medium effect size = 0.30 and large effect size = 0.50 (alpha = 0.05).

DISCUSSION

The present study revealed that the COMT val158met polymorphism affected the antidepressant effect of milnacipran. The met/met genotype of this polymorphism was associated with a significantly faster therapeutic effect in the MADRS scores during this study, although the difference in final therapeutic response was not significant between the met/met and other genotype groups.

Lachman *et al.* (1996) reported that individuals with the met/met genotype of the COMT val158met polymorphism had a threefold to fourfold reduction in enzymatic activity compared with those with the val/val genotype, and heterozygous individuals had intermediate enzymatic activity between that of homozygous individuals. However, the impact of the COMT val158met polymorphism on the metabolism of catecholamines appears to be minimal in usual physiological condition, even though it is a functional polymorphism. The high-affinity neuronal reuptake is an efficient elimination system for the released catecholamines, being responsible for most of their elimination both in the peripheral tissues and the brain (Mannisto and Kaakkola, 1999).

When exogenous levodopa, a dopamine precursor, is administered, the situation is dramatically altered for dopamine. During the combination therapy of levodopa and dopa decarboxylase inhibitor, the majority of surplus levodopa is preferably metabolised by COMT (Mannisto and Kaakkola, 1999). Individual differences of COMT activity become important for the pharmacological effect of levodopa in this situation.

The similar situation can occur to noradrenaline when its synaptic concentration is pharmacologically increased by the reuptake inhibition induced by milnacipran, though it has not been investigated yet. As the individuals with the met/met genotype of the COMT val158met polymorphism have a lower enzymatic activity, the synaptic concentration of norepinephrine may remain higher in patients with the met/met genotype than those with other genotypes. One possibility to explain the present result is that prolonged higher synaptic concentration of norepinephrine potentiates its neurotransmission particularly in patients with the met/met genotype, resulting in a faster antidepressant effect.

The present result about the COMT val158met polymorphism is not consistent with that of a previous study using an antidepressant mirtazapine (Szegedi *et al.*, 2005). Szegedi *et al.* (2005) reported that carriers of the val/val and val/met genotype had significantly greater antidepressant effect than those of the met/met genotype. The initial pharmacological action of milnacipran and mirtazapine is not identical: that of the former is blockade of noradrenaline transporters, and that of the latter is blockade of α_2-adrenergic autoreceptors. However, the discrepancy of the present results and those of Szegedi *et al.* (2005) cannot be explained by the difference of the initial pharmacological action of milnacipran and mirtazapine, because both drugs commonly result in enhanced noradrenergic transmission. Detailed mechanisms underlying the discrepancy of the present results and those of Szegedi *et al.* (2005) remain unclear.

Additional analyses based on the results of factor analyses of depression symptomatology revealed that the factor of dysphoria (Suzuki *et al.*, 2005) and psychic anxiety (Parker *et al.*, 2003) contributed to overall differences over time of the MADRS scores among each COMT val158met genotype group. The factor of dysphoria identified by Suzuki *et al.* (2005) and that of psychic anxiety identified by Parker *et al.* (2003) shares the symptoms of pessimistic and suicidal thoughts. Although serotonergic dysfunction in brain has been reported to be responsible for these symptoms (Carroll, 1994), this conclusion is not adequately justified by current evidence. For example, Poelinger and Haber (1989) found anxiety ratings decreased more with maprotiline (noradrenaline selective agent) than with fluoxetine (serotonin selective agent). Akkaya *et al.* (2006) reported that response rate for anxiety of reboxetine (noradrenaline selective agent) group was significantly higher than venlafaxine groups in the middle of treatment in patients with anxious depression, though the final response rate for anxiety was not significantly different. These findings and the present results suggest that the noradrenergic system in brain play a role in improvement of anxious symptoms of depression, and its genetic polymorphisms might affect the onset of therapeutic efficacy of milnacipran for anxiety in depression.

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The present study also revealed that the TH val81met polymorphism did not affect the antidepressant effect of milnacipran. The TH val81met polymorphism is reported to be associated with early-onset alcoholism (Dahmen *et al.*, 2005) and the left ventricular structure (Linhart *et al.*, 2002). However, Ishiguro *et al.* (1998) reported that TH val81met polymorphism was not likely to play a major role in the genetic predisposition to schizophrenia, mood disorders or alcohol dependence. Kunugi *et al.* (1998) also reported no evidence for involvement of the TH val81met polymorphism in schizophrenia or Parkinson's disease. The functional effect of the TH val81met polymorphism is still unknown, and the present results indicate no important role of the TH val81met polymorphism on the antidepressant effect of milnacipran.

One major limitation of this study is the relatively small number of subjects. A second limitation is the relatively small endpoint treatment differences in the analysis for the COMT val158met polymorphism. These limitations make it difficult to definitely conclude that the COMT val158met polymorphism is the genetic factor to predict the antidepressant effect of milnacipran. Difference in allele frequencies of the TH val81met polymorphism between responders and nonresponders seems marginal ($p = 0.08$), and increased number of subjects may reveal significant difference. Serotonergic effects of milnacipran cannot be neglected, and are probably independent of genetic differences in enzyme activities affecting catecholamine biosynthesis and elimination. Therefore, genetic polymorphisms of TH and COMT only have limited predictive value, and if any, can be at most partial predictors for the overall response to milnacipran. The authors performed collection of blood samples 4 weeks after initiation of antidepressant treatment. This schedule makes it impossible to perform an intent-to-treat analysis in relation to genetic polymorphisms, because the authors have no information of genotypes of dropout subjects. Further studies with a larger number of subjects are needed not only to confirm the results of this study but also to investigate the interaction of many genes, including the COMT gene, on the mechanisms of antidepressant action.

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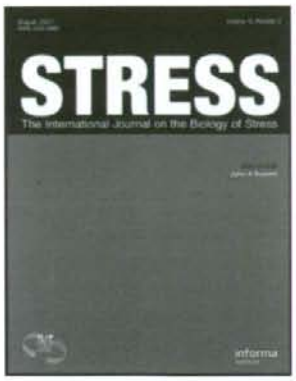

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Changes in brain tryptophan metabolism elicited by ageing, social environment, and psychological stress in mice

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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

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macrophages directly stimulates CRH release from the paraventricular nucleus (PVN) in the hypothalamus. Thus, hyperactivity in the hypothalamo-pituitary–adrenal (HPA) axis in major depression may be caused by macrophage hyperactivity. Furthermore, activated macrophages also relate to IDO activation as above-mentioned, and may inhibit brain 5-HT synthesis. Thus, this theory may explain both HPA-axis hyperactivity and 5-HT depletion, two main etiological and pathophysiological hypotheses of major depression (Konsman et al. 2002; Myint and Kim 2003).

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

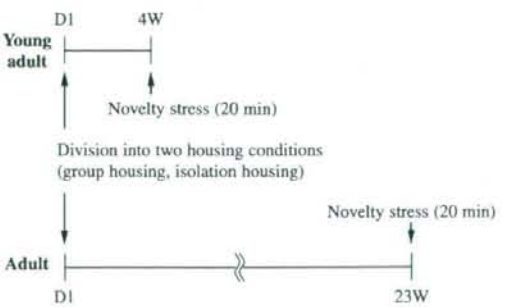

Figure 1. Time schedule of experiments. D: day; W: week.

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 μM isoproterenol (ISO) and 3-nitro-L-tyrosine (3-N TYR) as an internal standard per 100 mg of wet tissue. The solution was sonicated and then centrifuged at 10,000*g* for 20 min at 4°C. The supernatant was filtered through a Millipore HV filter (0.45 μm pore size) and then subjected to both high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) of 5-HT, and HPLC with fluorimetric detection (FD) of TRP and with ultraviolet (UV) detection of KYN. The standard solution was prepared using the above-mentioned ice-cold 0.2 M trichloroacetic acid solution containing 0.5 μM internal standards (ISO, 3-NTYR) and adjusted to 0.5 μM for 5-HT and KYN, and 10 μM for TRP.

HPLC determination of brain levels of 5-HT

The concentrations of 5-HT in the brain extracts were measured by HPLC with ECD. The system employed for HPLC–ECD consisted of a CMA/200 autosampler (CMA/Microdialysis AB, Stockholm, Sweden), a micro LC pump (BAS, West Lafayette, IN, USA), an LC-4C ECD (BAS), a Bio-Phase ODS-4 51-6034 column (4.0 × 110 mm; BAS), a CR-6A recorder (Shimadzu, Kyoto, Japan), an LC-26A vacuum degasser (BAS) and a CTO-10A column heater set at 35°C (Shimadzu). The mobile-phase solution consisted of 0.1 M tartaric acid–0.1 M sodium acetate buffer, pH 3.2, containing 0.5 mM EDTA-2Na, 555 μM 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