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Statistical mapping analysis of serotonin synthesis images generated in healthy volunteers using positron-emission tomography and α-[^{11}C]methyl-L-tryptophan

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Objectives: To assess the suitability of analyzing functional images of brain serotonin (5-HT) synthesis with statistical parametric mapping (SPM), and to investigate further possible sex-related regional differences. **Design:** Prospective study. **Participants:** Six healthy men and 5 healthy women. **Intervention:** Participants' brains were scanned with positron-emission tomography (PET) after intravenous injection of α-[^{11}C]methyl-L-tryptophan (α-[^{11}C]MTrp). **Outcome measures:** Tissue radioactivity images were converted into functional images using the Patlak plot approach, and analyzed with 2 methods for global normalization in the SPM program: proportional scaling and analysis of covariance (ANCOVA). **Results:** The data structure suggests that PET α-[^{11}C]MTrp data meet the criteria for analysis with SPM, and that the proportional scaling method is more appropriate than the ANCOVA method for normalization. Regional differences in 5-HT synthesis were identified between men and women, and the significance of these findings was supported by region of interest (ROI) analyses. **Conclusion:** SPM analyses of PET α-[^{11}C]MTrp data may be of value for identifying regional differences in brain 5-HT synthesis between groups, and in investigating the effects of psychotropic drugs. Since we found regional differences between male and female subjects, men and women should not be grouped for data analysis in PET α-[^{11}C]MTrp studies.

Objectifs : Évaluer la pertinence d'analyser des images fonctionnelles de la synthèse de la sérotonine (5-HT) dans le cerveau au moyen de la configuration paramétrique statistique (CPS), et étudier d'autres différences régionales possibles liées au sexe. **Conception :** Étude prospective. **Participants :** Six hommes en bonne santé et cinq femmes en bonne santé. **Intervention :** On a examiné le cerveau des participants par tomographie par émission de positrons (TEP) après leur avoir injecté par voie intraveineuse de l'α-[^{11}C]méthyl-L-tryptophane (α-[^{11}C]MTrp). **Mesures de résultats :** Les images de radioactivité tissulaires ont été converties en images fonctionnelles au moyen de la méthode fondée sur le tracé de Patlak et ana-

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Medical subject headings: brain; data interpretation, statistical; magnetic resonance imaging; serotonin; radioactive tracers; sex factors; tomography, emission-computed; tryptophan

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lysées au moyen de deux méthodes de normalisation globale du programme CPS : établissement de l'échelle proportionnelle et analyse de covariance (ANCOVA). **Résultats :** La structure des données indique que les données sur l'α-[^{11}C]MTrp obtenues par PET satisfont aux critères d'analyse par CPS et que la méthode de l'échelle proportionnelle convient mieux que la méthode ANCOVA pour la normalisation. On a défini les différences régionales de la synthèse de la 5-HT entre les hommes et les femmes et des analyses selon la région d'intérêt ont appuyé la signification de ces résultats. **Conclusion :** Les analyses par CPS des données α-[^{11}C]MTrp peuvent aider à identifier des différences régionales dans la synthèse de la 5-HT dans le cerveau entre des groupes et à étudier les effets de psychotropes. Comme nous avons constaté des différences régionales entre les sujets masculins et féminins, il ne faudrait pas regrouper les hommes et les femmes aux fins de l'analyse des données dans le contexte d'études de l'α-[^{11}C]MTrp par PET.

Introduction

The brain serotonin (5-HT) neurotransmitter system has been implicated in various cognitive and behavioural functions.[1] Neuropsychiatric disorders characterized by disturbances in these behaviours have been hypothesized to be related to disturbances in 5-HT neurotransmission.[2,3] To date, there have been few satisfactory methods for measuring aspects of 5-HT neurotransmission in the living human brain. Recently, however, α-[^{14}C]methyl-L-tryptophan (α-[^{14}C]MTrp) has been developed as a tracer for measuring 5-HT synthesis rates in the brain of living mammals.[4,5] In the past 2 years, this tracer has been used to measure 5-HT synthesis rates in the human brain with positron-emission tomography (PET) and (α-[^{11}C]MTrp),[6,7] by measuring the blood-to-brain clearance constant (named by some the "5-HT synthesis capacity").[8,9] The method is based on the unidirectional uptake of α-MTrp, which is transported into the brain. In rat brain it is converted, in part, to α-methyl-serotonin (α-M-5-HT).[4] Tissue radioactivity images of α-MTrp can be easily converted to functional images representing the regional rates of 5-HT synthesis in brain using the Patlak plot approach.[10,11] Together with the use of tracers recently developed to assess 5-HT_{1A} receptors,[12] 5-HT_{2A} receptors,[13] and 5-HT neuronal transporter sites,[14] this method has made possible a more detailed investigation of integrated 5-HT transmission in human brain.

In rat experiments we have shown that 5-HT synthesis, as measured with labelled α-MTrp, can be influenced by drugs in a different way in the terminals and cell bodies of serotonergic neurons without a significant influence on plasma levels of tryptophan.[15–19] In addition, in some experiments an increase was seen in 5-HT synthesis in some structures, while there was a decrease in others.

In this study, we tested the suitability of using statistical parametric mapping (SPM) to analyze 5-HT synthesis images, normalized to the global mean value, obtained in young healthy men and women. The SPM method of analyzing PET data was used to facilitate the identification of regional differences that were not predicted beforehand. The 5-HT synthesis images were obtained with α-[^{11}C]MTrp and an ECAT EXACT HR+ scanner (CTI/Siemens, Knoxville, Tenn.), which provides an image of the whole brain and has a better resolution than the one used in a previous study.[6] We also evaluated the respective advantages and disadvantages of using 2 different approaches (analysis of covariance [ANCOVA] and proportional scaling) in the SPM program to remove global differences between subjects.

Methods

Subjects

Six male and 5 female right-handed healthy volunteers aged 20 to 33 years old were studied. Psychiatric evaluations were conducted using the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders*, revised (DSM-III-R), non-patient version.[20] Exclusion criteria included evidence of a past or present axis-I or -II DSM-III-R diagnosis in the subjects or in their first-degree relatives, or any significant medical illness. The study was approved by the Research and Ethics Committee of the Montreal Neurological Institute and Hospital, and the Institutional Review Board of McGill University. All subjects gave written informed consent before the study.

PET procedures

The radiopharmaceutical α-[^{11}C]MTrp was prepared by the method reported by Mzengeza et al.[21] The study was carried out in the morning after an overnight fast (water

was allowed *ad libitum*). The day before the PET study, all subjects received a standard low-protein diet[6] to reduce variability in plasma concentrations of amino acids including tryptophan, which might be attributable to individual differences in diet. Following intravenous injection of up to 800 MBq (about 22 mCi, dose was not scaled to the weight of subject) of α-[^{11}C]MTrp over 2 minutes, 60-minute dynamic PET scans were started from the beginning of injection. Blood samples were drawn from an antecubital vein every 15 to 30 seconds in the first 2 minutes, every 1 minute during the next 3 minutes, every 3 to 5 minutes up to 20 minutes, and every 10 minutes for the remainder of the scan. The rationale for using the venous plasma samples for the input function is provided elsewhere.[7] Five additional venous samples were drawn for high-performance liquid chromatographic (HPLC) analysis to measure free and total plasma concentrations of tryptophan. PET scans were obtained with an ECAT EXACT HR + whole-body tomography scanner, which has 63 image slices at an intrinsic resolution of $5.0 \times 5.0 \times 5.0$ mm full width at half maximum (FWHM). All images were collected in a 3-dimensional mode. Images were reconstructed using Hanning filter of 8.1 mm FWHM in the transaxial direction. Before beginning the dynamic PET scans, transmission scans were performed using ^{68}Ga for attenuation correction.

Coregistration of PET and MRI

Each subject underwent high-resolution magnetic resonance imaging (MRI) scanning (160 slices, 1 mm thick) obtained with a Siemens Vision scanner (1.5 T, CTI/Siemens). Coregistration of individual PET and MRI images was performed using an automatic procedure[22] that uses averaged tissue activity images obtained between 5 and 60 minutes.[11] The MRI images from each subject were transferred into Talairach space[23] automatically.[24] Using parameters obtained from the automatic coregistration and transformation, the functional PET images were resampled linearly into the stereotaxic coordinates space of Talairach[11] and used for comparison between groups in the SPM program.

Estimation of the input function

In order to calculate 5-HT synthesis rates with venous samples, normalized input functions were estimated using the previously reported method.[7] Briefly, the time-radioactivity curves (TACs) obtained from the sinuses were normalized to the venous plasma radioactivity at 20 minutes to obtain the input function for the initial 20 minutes. After this time point, the time-radioactivity of venous plasma was used. To obtain the TACs from the sinuses, small regions of interest (ROI) were drawn at the slice level of the confluence of sinuses using 2 or 3 slices. The normalized venous sinus TACs were used as the input functions for the calculation of 5-HT synthesis rate images in all 11 subjects. As described previously,[7] the use of this input function results in values for the brain uptake constant that are not significantly different from those calculated with the arterial input function.

Calculation of functional images of 5-HT synthesis rate

The net plasma-to-brain uptake constant (K*, in mL/g per minute) for α-MTrp was calculated by using the Patlak plot method[10] applied to the data points after an apparent steady state was assumed.[7,25] Briefly, the kinetic model is based on the unidirectional uptake of α-MTrp, which is transported into the brain and shown, at least in part, to be converted in dorsal raphe to α-M-5-HT.[4] However, as originally described,[4] in this model it is sufficient that the tracer be transferred to an irreversible compartment, which would serve as a precursor for 5-HT synthesis. The existence of such a pool has been described by Mandel and Knapp.[26] In the Patlak plot method, tissue data in the form of distribution volume (DV, mL/g) were fitted as a function of exposure time (θ, min), and K* was determined using a linear portion of the plot (20 minutes to the end of the scanning time) assumed to correspond to the time after which an apparent steady state is achieved.[6,7] Patlak plots of PET α-[^{11}C]MTrp data obtained in our human studies have a reasonable linear portion, as can be seen in graphs presented previously by us[6,7,18] and by Muzik et al.[8] Why the plots reported by Shoaf et al[27] appear less linear than those reported by us[6,7,18] is unclear, but could be related to the use of anesthesic and the fact that the procedure was performed in a different species.

Functional images of K* were calculated pixel by pixel based on the Patlak plot method.[11] K* images were converted into images of 5-HT synthesis rate (R, pmol/g per minute) by the equation $R = K^* C_p^{\text{Free-Trp}} / LC$, where $C_p^{\text{Free-Trp}}$ stands for the plasma concentration of free tryptophan. A lumped constant (LC) of 0.42,

measured in vivo in rat brain,[28] was used to convert tissue uptake of tracer, α-[^{11}C]MTrp [K*], into tissue uptake of Trp [K(Trp)] via the 5-HT metabolic pathway. It needs to be emphasized that this division converts an experiment involving α-[^{11}C]MTrp to the experiment involving Trp, and only the part of Trp going into 5-HT synthesis. Then, the multiplication of calculated K(Trp) with $C_p^{Free\text{-}Trp}$ yields the regional 5-HT synthesis rate (R). Recently, the LC was also calculated through ex vivo measurements of the Michaelis-Menten constants of tryptophan hydroxylase for Trp and α-MTrp in the monkey brain and reported to be 0.18 ± 0.05.[29] However, in that study, the LC was calculated using the plasma concentrations of total Trp and α-MTrp instead of the plasma concentratons of free Trp and α-MTrp, whereas the plasma concentrations of free Trp and α-MTrp were used in the measurements of Vanier et al.[30] Although the LC may be species-dependent, we chose 0.42, the in vivo measured LC, as in our previous reports of human studies.[6,7,11] Regional 5-HT synthesis rates of 13 brain structures were obtained by the method previously described[11] and plotted as a function of the global rates (global rate in each subject was calculated as an average of all pixel values), to test for any putative relation. The same procedure was carried out to assess the relation between the regional and global K*. The errors associated with each pixel values were calculated[11] and confirmed to be normally distributed by using a standard normality test, meeting an important prerequisite for the use of *t*-tests and *z*-maps. In this investigation, 5-HT synthesis images were also calculated because they were needed for ANCOVA in the SPM normalization.

Statistical analysis

The functional PET data were analyzed with SPM 96,[30–33] developed and distributed by the Hammersmith Hospital (London, England). Functional image files after standardization into Talairach space were converted to the file format that can be applied to SPM program. Before SPM comparison, all images were smoothed using Gaussian filter (14 mm FWHM) to reduce the effect of the individual variability in cortical gyral anatomy of the brain. This procedure is recommended by the SPM authors. To remove the effect of global differences in regional values among subjects, 2 approaches were compared: proportional scaling and ANCOVA.[30] In the proportional scaling method, the functional images were normalized by the mean global values for synthesis (global mean = 50 pmol/g per minute, close to the mean 5-HT synthesis rate[6]) and, in the case of the K* constant, to 4×10^{-3} mL/g per minute. SPM comparisons were made at a threshold of 80% of the peak value.[30–33] The *t*-test was applied pixel by pixel to compare the regional differences in 5-HT synthesis images between men and women. The *t*-value for each pixel was then converted to a normal standard distribution (*z*-values), independent of the degree of freedom of the error, and this constituted a statistical parametric map, as reported by Friston et al.[31] To identify the regions considered to show a significant difference, 2 thresholds were used. First, the height threshold (*u*) used to interpret the *t*-test in terms of probability levels was set at $z = 2.33$ ($p = 0.01$), with each cluster including a peak of $z > 3.0$ ($\sim p < 0.001$). Second, the extent threshold (*k*) was set as 100 voxels (100 voxels corresponds to about 0.8 cm^3 of brain) to remove small clusters resulting from noisy voxels, which may have reached significance by chance.[33]

To confirm the significance of the SPM results, 3 regions were selected bilaterally, and ROIs were drawn at the same locations defined by the SPM analysis using the average MRI result in the Talairach space. R values were obtained from functional images after normalization of global R values,[11] and compared between men and women using the same ROIs.

Table 1: Characteristics of male and female healthy volunteers undergoing α-[^{11}C]methyl-L-tryptophan positron-emission tomography of the brain

	Mean (standard deviation)			
Characteristic	Men *n* = 6		Women *n* = 5	
Age	26.8	(5.2)	22.7	(3.8)
Free tryptophan, nmol/mL	4.5	(2.3)	4.6	(1.3)
Global K*, mL/g per minute	3.5×10^{-3}	(1.9×10^{-3})	4.7×10^{-3}	(2.3×10^{-3})
Global R, pmol/g per minute	43	(45)	53	(30)
Total tryptophan, nmol/mL	43.2	(8.1)	46.3	(4.7)

Plasma concentrations of free and total Trp, and regional 5-HT synthesis rates in the selected brain structures, were compared statistically by analysis of variance (ANOVA). Statistical significance was defined as $p < 0.05$.

Results

The plasma concentration of free and total Trp did not differ significantly (ANOVA, $p > 0.05$) between men and women (Table 1). There were linear relations with zero intercepts between global and regional 5-HT synthesis (Fig. 1A) and K* values (Fig. 1B), as illustrated by the 5 structures plotted in Fig. 1 as well as in the other 8 structures. The errors in the K* and R images were statistically evaluated, and analyses showed that these errors are normally distributed (the probability plots were linear in all subjects according to MATLAB evaluation). The ranges of global 5-HT synthesis were between 15.6 and 133.7 pmol/g per minute for men and between 10.5 and 78.9 pmol/g per minute for women. The global K* values were between 2.24×10^{-3} and 7.2×10^{-3} mL/g per minute for men and between 1.26×10^{-3} and 7.57×10^{-3} mL/g per minute for women. The mean values and respective standard deviations (SD) are given in Table 1. There were no significant (ANOVA, $p > 0.05$) differences in global R or K* between male and female subjects.

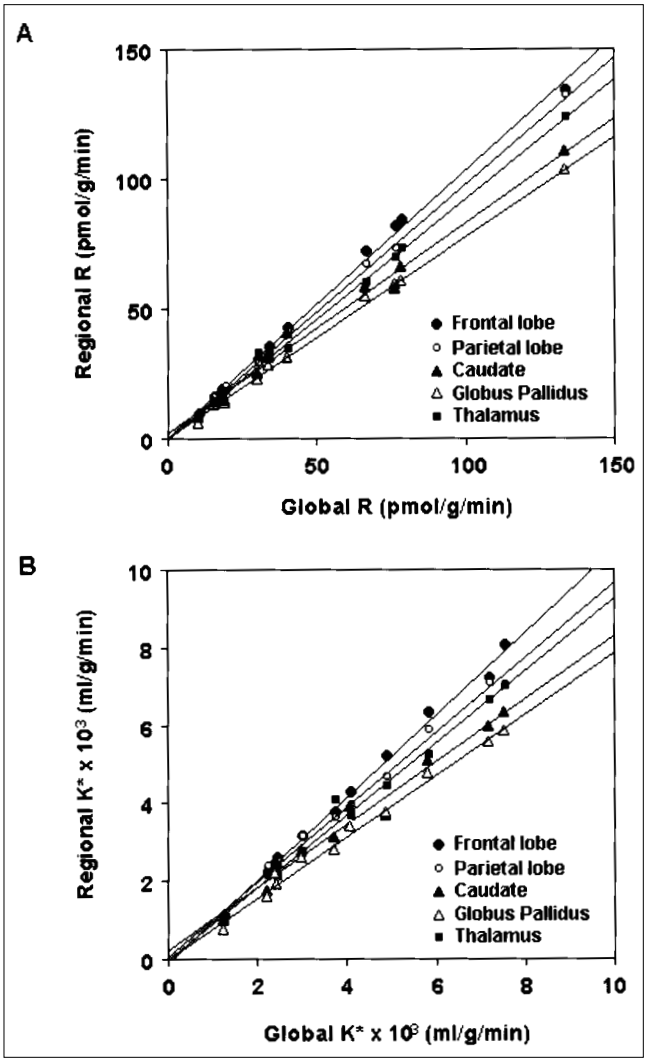

Fig. 1: The relation between regional and global 5-HT synthesis rates (R) and plasma-to-brain clearance constant (K*) in 5 brain structures. A linear regression line fits well to data in each structure. The relations between global and regional values are proportional, as required for the proportional scaling method in the statistical parametric mapping (SPM).[30]

Comparisons between healthy men and women according to SPM{Z} are depicted in Figs. 2 and 3 using the thresholds $z = 2.33$ ($p = 0.01$) and $k = 100$. Fig. 2 was obtained using proportional scaling (A) and ANCOVA (B) to remove global differences. With the proportional scaling method, regional rates of 5-HT synthesis were higher than the threshold in men compared with women in 6 regions: the right superior parietal lobe, the parieto-occipital region and the middle frontal gyri bilaterally, as well as the posterior part of the left rectal gyrus and the right posterior inferior temporal gyrus. Four of these regions did not reach the threshold for significance when analyzed using the ANCOVA method. The cluster centres and *z*-values are presented in Table 2. In the right parietal lobe, the greatest differences were seen in Brodmann's areas (BA) 7 and 40. In the frontal lobe, bilateral middle frontal gyri (BA 6) and the posterior part of left rectal gyrus (BA 25) in the orbitofrontal region showed higher 5-HT synthesis rates in men than in women. There were also differences in BA 19 in the left parieto-occipital areas and in BA 37 in the right posterior inferior temporal gyrus. The ANCOVA method provided lower *z*-scores than the proportional method for the corresponding structures detected as significant in the latter method. In Fig. 3 the sex-related differences in 5-HT synthesis in regions of the brain provided by the proportional scaling method with the same threshold are superimposed on the 3-dimensional brain image of Talairach.

Regional synthesis rates, as analyzed with the proportional scaling method, were higher in women compared with men in 4 regions in the right hemisphere: the putamen, the deep central sulcus (BA 4), the medial

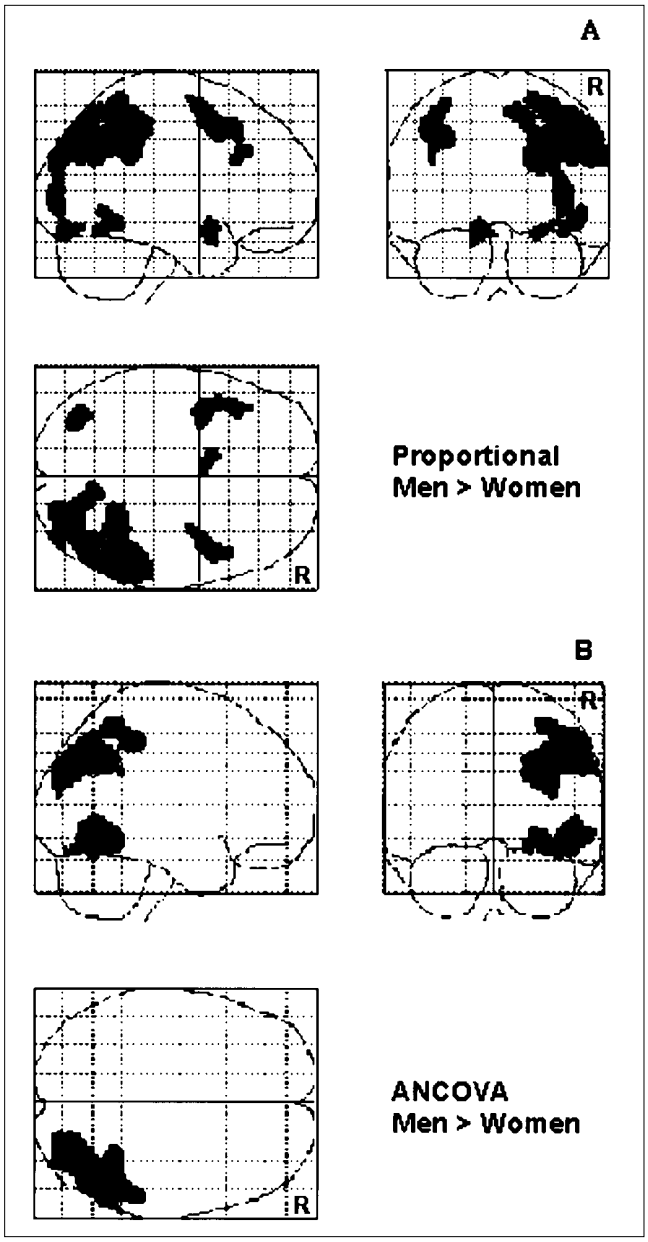

Fig. 2: Healthy volunteers (6 men and 5 women) are compared by SPM{Z}, using the threshold z = 2.33 (p = 0.01) and extent threshold k = 100 voxels. Significantly greater mean 5-HT synthesis in men than in women was demonstrated using proportional scaling (A) and analysis of covariance (ANCOVA) (B). In the proportional scaling method, there are significantly different rates of 5-HT synthesis in the following regions: right parietal lobe (Brodmann's area [BA] 7, BA 40), bilateral parieto-occipital lobe (BA 19), bilateral middle frontal gyri (BA 6), posterior part of right rectal gyrus (BA 25) and posterior part of right inferior temporal gyrus (BA 37); in the ANCOVA method, the significant differences are only seen in right parieto-occipital area and BA37. R = right side of the brain.

frontal lobe (BA 9-10) and the middle cingulate gyrus (BA 24). A cross-section at the level of basal ganglia shows that the greater 5-HT synthesis rates in women than in men are focused in the lateral portion of the right putamen (Fig. 3H). As above, not all of these differences met the required threshold when analyzed with the ANCOVA method (Table 2).

The same SPM analyses were carried out using K* images with proportional scaling method, in which the global mean of K* (4×10^{-3} mL / g per minute) was used for the global normalization. The results were exactly the same as those obtained with R images.

Three regions were selected bilaterally according to the results of SPM, and the regional values of 5-HT synthesis rates were obtained by the ROI method described above (Table 3). In these ROI analyses, there were significant differences in the parieto-occipital regions, middle frontal gyri bilaterally and the lateral portion of the right putamen (ANOVA, $p < 0.05$), while there was no significant difference in the left lateral putamen. These findings correspond to those obtained with SPM.

Discussion

The main objectives of this study were to assess the suitability of analyzing functional images of 5-HT synthesis with SPM, and to investigate further possible sex-related regional differences. These objectives overlapped. When data from this study are combined with those from our previous one, a bimodal distribution is observed similar to that seen in cerebrospinal fluid (CSF) concentrations of 5-hydroxyindolacetic acid (5-HIAA).[34,35] To the extent that CSF 5-HIAA concentrations might reflect, at least in part, 5-HT metabolic processes in the brain, including 5-HT synthesis, both CSF 5-HIAA measurements and PET α-[^{11}C]MTrp data suggest that there is considerable intersubject variability. This large spread of 5-HT synthesis within groups makes meaningful comparisons using ROI values very difficult. In this study, individual pixel values were normalized to the global values before regional differences were evaluated. This procedure increases statistical power.[30–33] In addition, the α-[^{11}C]MTrp data show large spread, similar to that reported earlier for the CSF 5-HIAA concentration data.[34,35]

Recently, some reports questioned the appropriateness of the α-MTrp method for measuring 5-HT synthesis, on the basis of experiments in anesthetized monkeys[27] and rats.[36] We have discussed these experiments

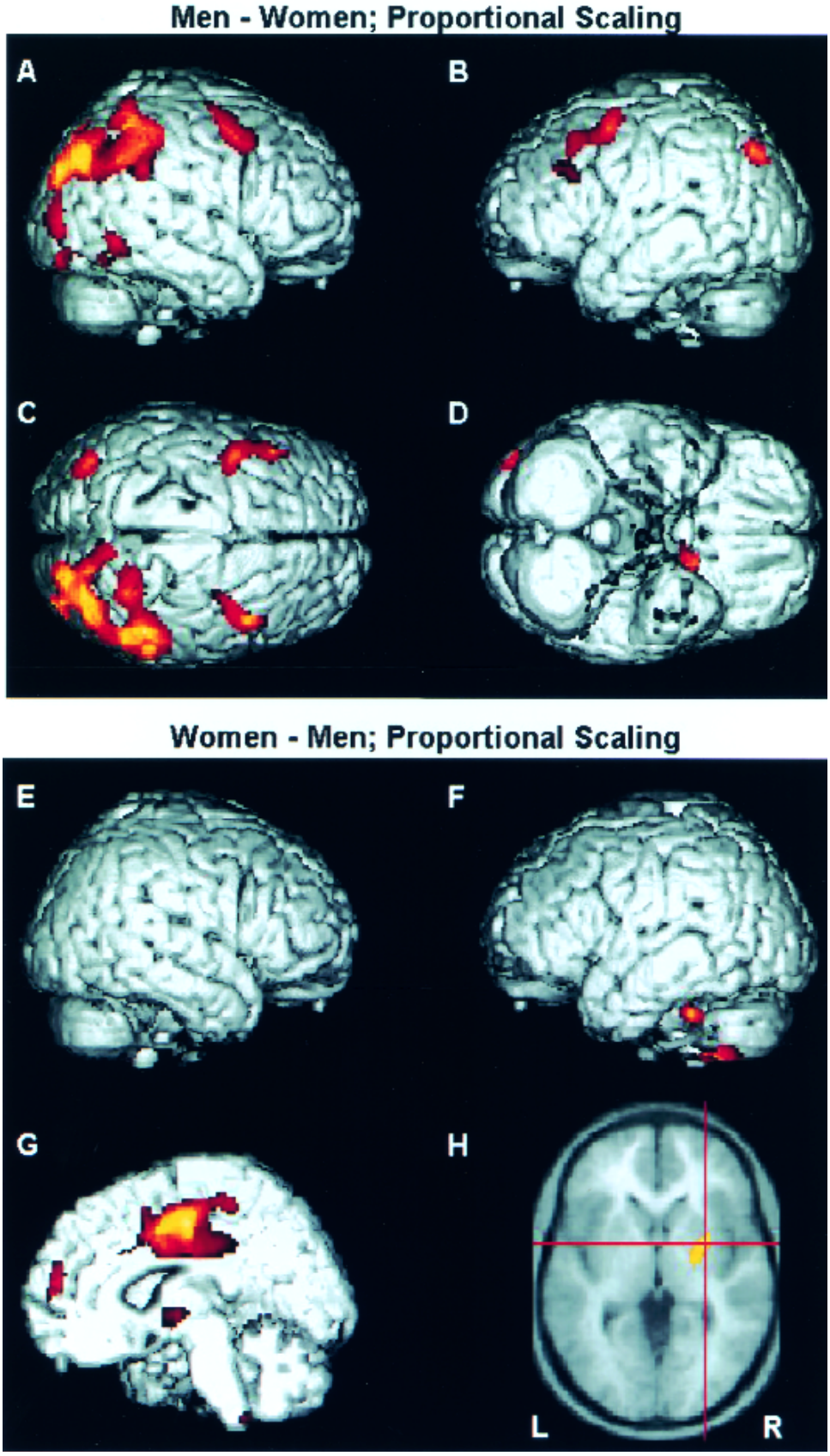

Fig. 3: Significantly different regions are superimposed on a 3-dimensional image of the Talairach brain. (A to D) Regions of higher 5-HT synthesis in men than in women. (E to G) Regions of higher 5-HT synthesis in women than in men. (A and E) show the right view, (B and F) show the left view and (G) shows the right medial view of the brain. (A to D) show the same areas as in Fig. 2A. (G) shows significant differences in the right medial frontal lobe and right middle cingulate gyrus. A cross-section (H) depicts the location of significantly greater 5-HT synthesis rate in women than in men (right putamen) superimposed on the average magnetic resonance image.

and the erroneous conclusions drawn from them elsewhere.[18,19] To further support our approach, experiments in rats have indicated that there is a highly significant linear relation between permeability surface (PS) area product, measured as proposed by Fenstenmaher,[37] for Trp and α-MTrp and have found a highly significant linear relation ($F = 48.9$; $p < 0.0001$) among 39 brain structures, with a slope of 0.96 (unpublished data). In addition, evaluation of the linear relation between normalized (normalized to dorsal raphe) PS product and 5-HT synthesis in our control rats shows that there is no significant relation (F test, $p > 0.2$). This suggests that trapping of this tracer is more complicated than simple brain–blood barrier transport. These results, in addition to our previous publications describing differential drug effects of 5-HT synthesis,[5,6,7,12–19] and the report that there is a correlation between tracer trapping and post mortem tissue concentration of 5-HT,[9] suggests that the tracer is appropriate for the measuring the rate of brain 5-HT synthesis.

SPM, compared with ROI analyses, is thought to be more powerful for identifying regional differences, in the presence of a global within-group variability (a confounding variable), because it removes global differences before statistical comparison. Until now, the SPM method has been used mainly for activation studies

Table 2: Location of significant ($z > 3.0$) differences in 5-HT synthesis between men and women

Area	Brodmann's area	Talairach coordinates x	y	z	z-score
Men > women, proportional scaling					
Right superior parietal lobe	7	30	–52	58	4.05
	40	52	–40	46	3.54
	40	60	–54	36	3.42
Right occipitotemporal lobe	37	48	–52	–8	3.16
Right middle frontal gyrus	6	48	14	48	3.14
Left middle frontal gyrus	6	–40	4	52	3.11
Left posterior rectal gyrus	25	–12	6	–14	3.10
Left superior occipital gyrus	19	–38	–74	40	3.08
Women > men, proportional scaling					
Right putamen		28	2	–2	3.83
Right deep central sulcus	4	24	–26	34	3.53
Right medial frontal lobe	9–10	2	54	18	3.38
Right cingulate gyrus	24	10	–2	38	3.06
Men > women, ANCOVA					
Right superior parietal lobe	40–19	44	–70	40	3.61
	40	34	–50	54	3.22
Right occipitotemporal lobe	37	48	–62	–4	3.43
Right superior occipital gyrus	19	36	–74	34	3.26
Women > men, ANCOVA					
Right putamen		28	–2	0	3.43
Right deep central sulcus	4	22	–14	46	3.81

Table 3: Regional 5-HT synthesis rates in the areas of significant difference

Region	Regional 5-HT synthesis rate [R], mean (and SD) after global normalization (i.e., global mean = 50 pmol/g per minute) Men	Women	*p* value (ANOVA)
Right parieto-occipital region	49 (3)	40 (7)	0.01
Left parieto-occipital region	52 (3)	46 (4)	< 0.05
Right middle frontal gyrus	52 (3)	46 (5)	< 0.05
Left middle frontal gyrus	49 (2)	43 (5)	< 0.05
Right putamen	44 (4)	50 (4)	< 0.05
Left putamen	51 (4)	51 (3)	> 0.9

with ^{15}O-water PET or functional MRI studies;[30–33,38] however, the use of SPM has recently been extended to mapping histamine H_1 receptors,[39] 6-[^{18}F]fluorodopa[40] and 2-[^{18}F]fluoro-deoxyglucose.[41] Since this study is the first to apply SPM analysis to compare 5-HT synthesis rate images, we also conducted basic assessment of the image data and confirmation of the regional differences.

First, we previously described a method for making functional images using the Patlak plot approach, and their transformation into the stereotaxic coordinate space of Talairach to improve and simplify image manipulation.[11] K* values obtained from the fit of the data to the full operational equation and those obtained by the Patlak plot approach are not significantly different.[11] This enables the production of functional images for mathematical analyses using brain mapping programs, which makes comparisons easier and removes possible systematic errors (e.g., observer error). Second, in this study, we used a brain mapping program, SPM analysis. As noted, SPM analyses have the advantage of removing inter-subject variability and standardizing images to the Talairach space, the procedure that facilitates comparisons of regional differences.

We compared 2 methods of removing the effect of global differences in regional values among subjects: proportional scaling and ANCOVA.[30] In the 5-HT synthesis images, the relations between regional and global values are proportional, and the regression lines have different slopes with zero intercepts (Fig. 1), as required by the proportional scaling method in SPM,[30] suggesting that the proportional model is more appropriate. Fig. 1 also illustrates that, within the same individual, values vary from region to region. This means that regional differences can be compared after a global normalization. The same areas were found to be significantly different when SPM analyses were performed with either R or K* images using the proportional method. The advantage of the proportional method is that all the constants or absolute values are cancelled by the proportional normalization. In comparison, the same results would not be obtained if one analyzed radioactivity images (unpublished data).

When ANCOVA was used to remove global differences in the SPM analysis, fewer differences and lower *z*-values were found than those obtained with the proportional method (Table 2). This result is consistent with the report of Chmielowska et al,[42] in which the authors compared 3 statistical methods: proportional scaling and ANCOVA in SPM, and the pooled variance method described by Worsley et al.[43] This finding is not unexpected because, as shown in Fig. 1, the requirements for the proportional scaling in the SPM are fully met, while requirements for the ANCOVA[30] are not met in the structures examined in the present data set. Based on these results, we propose that the proportional scaling approach with SPM might provide an appropriate method to evaluate regional differences in 5-HT synthesis between groups.

The threshold for significance was set with $z > 3.0$ at peak for the height and $k > 100$ for the extent. In regional cerebral blood flow (rCBF) activation studies, an uncorrected p value of less than 0.001 ($z = 3.09$)[44] or a theoretically corrected threshold for multiple comparisons[32] is used to determine significantly activated areas for the removal of a few voxels that may reach significance by chance. However, in the α-MTrp study, the image contrast is poorer than in rCBF images obtained with ^{15}O-water, and there has been no previous report comparing the 5-HT synthesis images using SPM. The threshold for significance should be carefully selected, because there is a possibility of obtaining false-positive or false-negative regional differences. To confirm that the SPM findings of significance are correct, 3 regions were selected bilaterally at the same coordinates as reported by the SPM, and the values of the ROIs were obtained from the 5-HT synthesis rate images after global normalization. The same ROIs in the Talairach space were used for all the functional images.[11] In the bilateral parieto-occipital region and middle frontal gyri, 5-HT synthesis rates were significantly higher in men than in women, whereas the value of the right putamen in females was significantly greater than in males. This finding was in good agreement with the results of the SPM analysis using proportional scaling. This is not surprising, because the ROI method used here to confirm the significant differences is basically the same approach as the proportional scaling method in the SPM.

Sex-related differences in aspects of 5-HT neurotransmission have been suggested by researchers using other methods. For example, CSF concentrations of 5-HIAA have been reported to be higher in women than in men.[45,46] Our findings with the PET α-MTrp method suggest that sex-related differences in 5-HT metabolism may bear some regional specificity, and rates of synthesis might be higher in women than in men in some areas but lower in others. Recent functional neuroimaging and post mortem studies also suggest regionally

specific sex-related differences in 5-HT function.[47,48] The relevance of our findings to vulnerability to putative 5-HT-related pathologies is not clear. Although sex-related differences in the assignment of psychiatric diagnoses are commonly reported (e.g., higher rates of major depression in women[49]), this might also reflect the diagnostic criteria rather than the underlying pathology.

In our previous study, rates of brain 5-HT synthesis were 40% to 50% higher in men than in women,[6] but there was also a difference in the plasma concentration of free Trp, which could in part be responsible for the observed differences. This agrees with the work of many investigators who reported that an increase in the plasma concentration of free Trp results in an increase in brain 5-HT synthesis in rats,[50] and that the plasma concentration of free Trp is related to brain 5-HT synthesis.[51] Since tryptophan hydroxylase is not normally saturated with Trp, one would expect an increase in the synthesis of 5-HT when plasma Trp is increased. In this study, this difference was not found in a separate group of subjects scanned with a scanner with different characteristics. When data from both studies were combined, a bimodal distribution of brain 5-HT syntheses in male and female subjects was observed. The appearance of a bimodal distribution in both men and women is similar to the bimodal distribution of 5-HIAA in CSF.[34,35] Bimodal distributions greatly complicate group comparisons, but the difficulty can be diminished by conducting analyses, as described here, after removing global differences. However, we wish to emphasize that bimodal distribution or a large spread in brain 5-HT synthesis might be biological reality. The significant global differences between male and female subjects found in our first study may have reflected sampling from different portions of a bimodal distribution.

Conclusion

We analyzed functional images of 5-HT synthesis rates obtained by α-[11C]MTrp PET were analyzed with the SPM 96 program, and identified brain structures that showed sex-related differences in 5-HT synthesis. This suggests that groups of male and female subjects should be analyzed separately. This study also suggests that the SPM analyses are suitable for detecting small regional differences in rates of 5-HT synthesis, and might prove effective and robust for comparing relatively small groups. The analyses presented here suggest that proportional scaling is the most appropriate way to remove (normalize) within-group global differences in 5-HT synthesis data. The procedure described here might be useful in clinical studies for identifying regional differences in rates of 5-HT synthesis between patients and healthy controls, and could be applied to the evaluation of individual or group differences in subjects treated with serotonergic drugs.

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CANADIAN COLLEGE OF NEUROPSYCHOPHARMACOLOGY
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