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DOI:

[10.1016/j.neuroimage.2018.10.013](https://doi.org/10.1016/j.neuroimage.2018.10.013)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Laurikainen, H., Tuominen, L., Tikka, M., Merisaari, H., Armio, R-L., Sormunen, E., Borgan, F., Veronese, M., Howes, O., Haaparanta-Solin, M., Solin, O., & Hietala, J. (2018). Sex difference in brain CB1 receptor availability in man. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2018.10.013>

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PII: S1053-8119(18)31972-4

DOI: [10.1016/j.neuroimage.2018.10.013](https://doi.org/10.1016/j.neuroimage.2018.10.013)

Reference: YNIMG 15332

To appear in: *NeuroImage*

Received Date: 28 February 2018

Revised Date: 20 September 2018

Accepted Date: 4 October 2018

Please cite this article as: Laurikainen, H., Tuominen, L., Tikka, M., Merisaari, H., Armio, R.-L., Sormunen, E., Borgan, F., Veronese, M., Howes, O., Haaparanta-Solin, M., Solin, O., Hietala, J., METSY group, Sex difference in brain CB1 receptor availability in man, *NeuroImage* (2018), doi: <https://doi.org/10.1016/j.neuroimage.2018.10.013>.

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Sex difference in brain CB1 receptor availability in man

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Abstract

The endocannabinoid system (ECS) has a widespread neuromodulatory function in the central nervous system and is involved in important aspects of brain function including brain development, cortical rhythms, plasticity, reward, and stress sensitivity. Many of these effects are mediated via the cannabinoid CB1 receptor (CB1R) subtype. Animal studies convincingly show an interaction between the ECS and sex hormones, as well as a sex difference of higher brain CB1R in males. Human *in vivo* studies of sex difference have yielded discrepant findings.

Gender differences in CB1R availability were investigated *in vivo* in 11 male and 11 female healthy volunteers using a specific CB1R tracer [¹⁸F]FMPEP-d2 and positron emission tomography (PET). Regional [¹⁸F]FMPEP-d2 distribution volume was used as a proxy for CB1R availability. In addition, we explored whether CB1R availability is linked to neuropsychological functioning.

Relative to females, CB1R availability was on average 41% higher in males ($p=0.002$) with a regionally specific effect larger in the posterior cingulate and retrosplenial cortices ($p=0.001$). Inter-subject variability in CB1R availability was similar in both groups. Voxel-based analyses revealed an inverse association between CB1R availability and visuospatial working memory task performance in both groups ($p<0.001$).

A CB1R sex difference with a large effect size was observed and should be considered in the design of CB1R-related studies on neuropsychiatric disorders. The behavioural correlates and clinical significance of this difference remain to be further elucidated, but our studies suggest an association between CB1R availability and working memory.

Key words: endocannabinoid, cb1 receptor, sex difference, working memory

1 INTRODUCTION

The endocannabinoid system (ECS) is a ubiquitous modulatory and homeostatic system in the human body¹. In the central nervous system, the ECS is involved in multiple neurodevelopmental processes², cognitive functions³, and the regulation of emotion and responses to stress⁴. The ECS consists of lipid derived endocannabinoid ligands, the enzymes constituting their metabolic routes, and two G-protein coupled receptors - endocannabinoid receptor type 1 and 2 (CB1R and CB2R respectively). The main endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are synthesised on the postsynaptic membrane in response to depolarization. They modulate neural activity by acting retrogradely on presynaptic CB1R to transiently suppress further neurotransmitter release. Additionally, the ECS can affect synaptic plasticity through long-term depression of function (LTD)⁵. The main functional site of ECS modulation has been proposed to be at glutamatergic and GABAergic synapses resulting in balancing excitation and inhibition of cortical pyramidal cells, and local circuit modulation of midbrain projections.⁵⁻⁷ It is currently unclear whether these roles of ECS in plasticity and neurodevelopment have an impact on known sex differences in histology and morphology of the human cortex^{8,9}.

Converging lines of evidence from pre-clinical studies show gender differences in ECS function. Relative to female rodents, male rodents show greater levels of CB1R protein¹⁰, mRNA¹¹, receptor density^{12,13} and agonist binding¹⁴. However, *in vivo* studies investigating CB1R availability in humans have yielded discrepant findings, reporting decreased and increased CB1R availability in males relative to females¹⁵⁻¹⁷. These widespread or global differences were measured in comparable samples of healthy control subjects without recent cannabis exposure, but unfortunately comparison is hampered by differences in quantification and properties of CB1R PET radioligands¹⁸.

Development of the ECS can be described as transactional. Pre-clinical studies showing ECS sex differences in response developmental stress exposure suggest that environmental factors may contribute to different adult ECS phenotypes in males and females^{10,13,19}. Additionally, chronic exposure to a CB1R agonist during early development results in long term sex dependent changes in cognitive performance in

rats¹². This is in line with human data suggesting greater impact of chronic cannabis exposure to visuospatial memory performance in females²⁰. Human studies generally associate the ECS with cognition²¹. In a recent double blind placebo controlled crossover study a low dose of sublingual $\Delta 9$ -THC resulted in reduction of spatial span task errors in males, and an increase in females²². This sex dependent effect of the ECS on visuospatial performance is notable as this domain seems to have sex differences in humans²³. Animal studies consistently support the association of visuospatial memory and the ECS^{24–29}; e.g. spatial working memory is selectively impaired in male rhesus monkeys after i.v. $\Delta 9$ -THC injection³⁰.

CB1R availability in the human brain is proportional to tracer binding measured as tracer distribution volume (V_T) using compartmental modelling³¹. V_T is defined as the relationship of tissue concentration of bound radioligand (C_T) to plasma concentration of unchanged radioligand (C_P) at kinetic equilibrium³². We used [¹⁸F]FMPEP-*d2*, a novel inverse agonist CB1R PET radioligand, to measure CB1R availability in healthy male and female volunteers. The retest variability of [¹⁸F]FMPEP-*d2* plasma measurements are good (16%), uptake into the human brain is high, and binding is highly specific (80-90%). Similarly, the test-retest variability (14%), intersubject variability (26%), and intra-class correlation (ICC=0.89) of [¹⁸F]FMPEP-*d2* V_T are good^{33,34}. Despite being highly lipophilic the combination of [¹⁸F]FMPEP-*d2* test-retest stabilities of V_T /plasma measurements and binding specificity seem to be superior to other currently available CB1R PET radioligands^{16,33,35–37}.

Previous studies have successfully used [¹⁸F]FMPEP-*d2* V_T to demonstrate group effects of chronic cannabis use and alcohol dependence on CB1R availability^{38,39}. In addition to substance-related disorders, the ECS has also been suggested to be involved in the etiology of other psychiatric disorders, such as mood and anxiety disorders^{15,40}, and schizophrenia^{41,42}. The clinical use of CB1R antagonist rimonabant increased the risk for significant depressive symptoms in patients with obesity/metabolic disorders⁴³ leading to withdrawal from market. Considering the consistent gender differences in incidence of these psychiatric disorders, and the widespread role of ECS in brain function, characterizing a ECS sex difference could lead to novel insight into the vulnerability mechanisms of neuropsychiatric disorders. We hypothesized that males would show greater CB1R availability relative to females

using [^{18}F]FMPEP-*d2* and 3D-PET. We also explored whether CB1R is associated with neuropsychological function.

2 MATERIALS AND METHODS

The study protocol was approved by the Joint Ethical Committee of the University of Turku and the Turku University Central Hospital. The study was conducted according to the Declaration of Helsinki. Informed consent was obtained from all human subjects prior to their participating in the study.

2.1 Subjects

We recruited 28 healthy individuals from the national population registry, local educational institutions, and by local newspaper advertisement. The somatic status of all the subjects was confirmed by medical examination, blood and urine tests, electrocardiography, and a structured clinical interview for DSM-IV axis I disorders (SCID-I/NP). Pregnancy was ruled out by urine and/or blood screening. Subjects with a chronic medical or neurological condition affecting the brain, history of head trauma with loss of consciousness, and neurodevelopmental disorders were excluded. Lifetime substance use was documented, and current use was controlled with a urine screen prior to the PET scan. Subjects with a lifetime DSM-IV Axis I diagnosis, substance-related disorder, or who had used any illicit substances two months prior to scanning, were excluded. All subjects underwent a structural MRI scan with the Philips 3T Ingenuity PET/MR hybrid scanner to exclude any structural abnormalities. Four subjects were ruled out from statistical tests due to technical failure resulting in incomplete blood data ($n=3$) or incomplete PET data ($n=1$). Two subjects with frame-by-frame motion exceeding 4 mm, and significant or continuous motion in tracking data, were excluded. Overall, the study sample regarding statistical tests of [^{18}F]FMPEP-*d2* V_T consisted of 22 of the 28 scanned subjects.

To approximate serum estradiol concentration during PET, the phase of ovarian cycle of females was determined as either early follicular (<9 d), late luteal (>24 d), or late follicular to mid-luteal phase (9-23 d) according to days from start of last menstruation⁴⁴. Use of hormonal contraception or menopause were documented as these affect serum estradiol levels⁴⁵. Six females had combined oral contraceptives containing estrogen and progestin, four had no contraception and one subject had a

recent menopause. According to ovarian cycle no subjects without contraception was in the 'late follicular to mid-luteal' phase. The cycle phase for one female subject could not be classified due to missing data.

2.2 Radiochemistry

[¹⁸F]FMPEP-*d*2 was synthesized as described previously⁴⁶ with slight modifications. The radiochemical purity was greater than 95% and the molar radioactivity greater than 500 GBq/μmol at the end of synthesis.

2.3 Positron emission tomography

An individually molded thermoplastic mask was used to restrain head movement during the scan. The tracer [¹⁸F]FMPEP-*d*2 was given as an antecubital intravenous bolus injection. Emission data was gathered in 3D list-mode first for 60 minutes with the brain-dedicated high-resolution research tomograph (ECAT HRRT, Siemens Medical Solutions). Subjects then came out of the scanner for 30 minutes, after which the scan was continued for another 30 minutes for a total scan range of 0 to 120 minutes.

Emission data were reconstructed using a 3D-OSEM algorithm into 19 frames of increasing length (3x1 min, 5x3 min, 7x6 min and 4x7.5 min) with a 1.22x1.22x1.22 mm³ isometric voxel-size. Head motion during the scan was corrected by realigning all PET frames to the 12th frame containing the highest uptake on average. Individual frame-to-frame motion correction parameters were inspected and subjects with motion exceeding a predefined 4mm in any direction were scrutinized using head motion tracking data collected during the scan (Polaris, Northern Digital Inc., Canada). Cortical reconstruction and volumetric segmentation of the T1 weighted MR image were performed with Freesurfer version 5.3.0 (<http://surfer.nmr.mgh.harvard.edu/>). The T1 weighted image, now in the same space as the freesurfer atlas, was coregistered to the sum of the realigned PET frames using SPM12 and Matlab R2014b (The Mathworks Inc., Sherborn, Massachusetts) and the same transformations were also applied to the Desikan-Killiany atlas in alignment with the T1 weighted image. This atlas was then used to extract masks to define volumes of interest (VOI) on the PET time series. To facilitate cross study comparison of [¹⁸F]FMPEP-*d*2 V_T, VOI selection was a rough adaptation from a

previous [^{18}F]FMPEP-*d2* human study³⁸, and mimicked the exhaustive or composite VOIs used in previous human studies of CB1R sex difference¹⁵⁻¹⁷. Mean time activity curves were derived from all 17 VOIs (amygdala, anterior cingulate cortex, brainstem, caudate nucleus, cerebellum, frontal cortex, hippocampus, insula, occipital cortex, orbitofrontal cortex, parahippocampal gyrus, parietal cortex, posterior cingulate cortex, prefrontal cortex, putamen, temporal cortex and thalamus).

2.4 Arterial plasma sampling and analyses for input function

Following tracer injection, whole blood activity was measured using continuous sampling for the first 3.5 minutes using Allogg ABSS (Allogg AB, Mariefred, Sweden, <http://www.allogg.se/>), after which manual samples were drawn from a radial artery cannula at 4.5, 7.5, 11, 15, 20, 25, 30, 35, 40, 45, 50 and 60 minutes. Plasma metabolite samples were also manually drawn from the radial artery at 4.5, 11, 15, 20, 30, 45 and 60 minutes. The amount of unchanged [^{18}F]FMPEP-*d2* and its radioactive metabolites were analysed from arterial plasma samples using thin-layer chromatography and digital autoradiography; see supplementary methods for details.

2.5 Plasma input curve preprocessing

Decay corrected whole blood tissue activity curves (TAC) derived from automatic blood pump sampling were converted to plasma activity using hematocrit and a population derived tracer specific distribution function. Automated and manual plasma sample TACs were then combined. Plasma TAC values were extrapolated from 60 to 120 minutes using a biexponential function fit starting at two times the peak activity location. The measured and estimated plasma activities were corrected for the fraction of unchanged tracer, which was interpolated with a Hill-function fit to the measured un-metabolized fraction time series. The time delay of the peak radioactivity reaching tissue and blood samples was corrected using PET count rate curves to reference of peak tissue activity. The resulting plasma activity concentration curve, corrected for metabolites, was used as parent input for modeling.

2.6 Assessment of confounding factors in blood data

To assess sources of variation in V_T , statistical tests of blood data were done with sex as the independent variable. Areas under curve (AUC) for parent input and unchanged

tracer fraction time series were calculated with the linear trapezoidal method using GraphPad Prism version 6.00 (GraphPad Software, La Jolla California USA, www.graphpad.com). Sex differences of whole parent input AUC means were compared with Student's t-test. Repeated measures analysis of variance (rmANOVA) within the general linear model framework (GLM) was used to test for a significant group effect or time*sex interaction in unchanged tracer fractions. Associations of blood data to body mass index (BMI) and age were explored by calculating Pearson correlation coefficients. The sources of variance in parent input were modeled with linear regression using body surface area (BSA), injected activity and AUC of unmetabolized tracer fraction as independent variables.

2.7 Tissue data characterization

Individual tissue time activity curves (TTAC) were visually inspected for quality. There were no significant hemispheric differences of regional V_T ($df=1$, $F=0.563$, $p=0.461$) in an rmANOVA model including all 17 ROIs and two hemispheres as within-subject factor levels. V_T from VOIs containing both hemispheres from the amygdala, anterior cingulate cortex, brainstem, cerebellum, frontal cortex, hippocampus, insula, nucleus caudatus, occipital cortex, orbitofrontal cortex, parahippocampal cortex, parietal cortex, posterior cingulate cortex, prefrontal cortex, putamen, temporal cortex and thalamus were chosen for further statistical testing.

2.8 Modeling of VOI V_T and subgroup comparisons

In this study regional [^{18}F]FMPEP-d2 V_T was calculated using Logan plot⁴⁷, a multiple time graphical analysis method, with fit start fixed at 42 minutes. Logan plot V_T has been shown to produce similar regional retest variabilities (13-18%) and ICCs (.85-.93) as 2-tissue compartmental modeling (9-17% and .85-.94 correspondingly), and is highly correlated ($R^2=.96-.98$) with 2-tissue compartmental model derived whole brain V_T ⁴⁷. Logan plot adds minor underestimation bias due to noise and provides robust measurements of V_T at both regional and voxel level⁴⁸. A substantial proportion of CB1R has been found to be internalized behind the neuronal plasma membrane⁴⁹. The proportion of internalized receptors might vary between brain regions. Logan plot permits not assuming a uniform model structure for the whole brain. Modeling was done with in-house developed software, which is freely available

for download online (<http://www.turkupetcentre.net/software/>). Shapiro-Wilks test was used to test the normality assumption. Correlations with subject age and BMI were assessed with Pearson's r correlations. Repeated measures ANOVA was used to test for differences in V_T between sexes. Greenhouse-Geisser correction was used when sphericity assumption was violated. Results from rmANOVA were reassured by leaving out thalamic and caudate nucleus VOIs in which the normality assumption was violated. Group differences in these two VOIs were tested with Mann-Whitney U-test. P-values <0.05 were considered statistically significant. All statistical testing was done with IBM SPSS Statistics 23 software (IBM corp. Armonk, NY, USA).

2.9 Voxel-wise modeling and statistical testing of V_T

Parametric images of V_T were calculated to discover the maximal sources of variance within regions, and to reassure results from VOI tests. First, the skull signal was masked out to minimize the effect of Gaussian filtering activity from skeletal [^{18}F]fluoride accumulation onto the cortical signal. The motion corrected PET images were then Gaussian filtered to the approximate spatial resolution (FWHM 3 mm) of HRRT to increase signal to noise ratio. Freely available in-house software was used to calculate voxel-wise V_T (<http://www.turkupetcentre.net/software/>). Modeling was started from the frame starting at 42 minutes without V_T constraints. The parametric volume was then Gaussian filtered (FWHM 10 mm) and normalized to standard MNI space using transformations obtained by aligning the T1 weighted MR image to a standard MNI template using SPM12. Concordance of VOI level V_T from the prefrontal cortex and putamen were tested against mean V_T from corresponding areas of MNI normalized parametric volumes using Pearson r correlations. An independent sample t-test was done with SPM12 to compare groups at voxel level. The threshold of statistical significance used in the VOI level tests of sex difference ($p < 0.05$) was used as peak threshold for corresponding parametric t-tests. In exploratory analyses, the peak threshold was set at $p < 0.001$. In all parametric analyses the FDR corrected cluster threshold was $p < 0.05$ and the extent threshold was adjusted to the size of the smallest significant cluster.

2.10 Neuropsychological testing

All subjects participated in neuropsychological testing including Trail Making Test A (TMT-A), Brief Visuospatial Memory Test (BVMT), a visuospatial working memory task (Spatial Span), word fluency, Hopkins Verbal Learning Test (HVLT) and Continuous Performance Test (CPT-IP). Gender differences in performance were tested for each test separately using univariate GLM including age as a covariate.

3 RESULTS

All subjects were of Finnish ancestry. Males and females did not differ in age, years of education, level of functioning, smoking status, handedness, average movement during PET scan or injected tracer activity; table 1. Additionally, there were no differences in self-reported anxiety (Beck Anxiety Inventory). Males had a larger body surface area than females ($df=20$, $t=4.496$, $p<0.001$), but there was no difference in BMI ($p=0.250$). There were no significant correlations between age ($p>0.150$) or BMI ($p>0.180$) and V_T in any VOI, with or without controlling for sex. There were no significant differences of V_T between hemispheres ($df=1$, $F=0.563$, $p=0.461$). Therefore, V_T from bilateral VOIs were used in statistical models. Results of statistical tests of blood data, and comparison of parametric and ROI V_T , can be found in supplement 2. A figure showing the time series of mean un-metabolized tracer fractions in males and females separately can be found in supplement 3.

Table 1 Demographic, clinical and imaging information of study groups

	Male	Female	P-value
Number of subjects (N)	11	11	
Right-handed (N)	11	11	
Age (years)	27±6	28±10	.72
BMI (kg m ⁻²)	25±4	24±3	.25
Years of education	16±3	16±3	.95
SOFAS	91±5	93±5	.36
Tobacco smokers/non-smokers (N)	1/10	1/10	
AUDIT score	9±6	5±3	.23
Lifetime cannabis use >5 times (N)	3	0	.214
Past year cannabis use <6 times (N)	3	0	.214
Injected [¹⁸ F]FMPEP-d2 activity (MBq)	200±13	205±13	.36
Injected [¹⁸ F]FMPEP-d2 mass (ng)	<189	<194	
BSA (m ²)	2.0±.14	1.8±.12	<.001
Parent plasma input AUC (kBq/ml*min)	132±32	188±25	<.001
Un-metabolized tracer fraction AUC (1*min)	1613±179	1918±417	.08
Average frame-to-frame movement (mm)	.42±.17	.45±.13	.30

Values are number, or mean±standard deviation. Abbreviations: AUC, area under curve; AUDIT, alcohol use disorders identification test; BMI, body mass index; BSA, body surface area; SOFAS, social and occupational functioning assessment.

3.1 Region of interest analyses of CB1R V_T in males and females

The V_T of [¹⁸F]FMPEP-d2 was higher in males compared to females (df=1, F=13.150, p=0.002), in a regionally differential manner (VOI*sex interaction: df=2.528, F=7.114, p=0.001). The V_T of males was significantly higher (p<0.05) in all studied brain regions. The largest effect sizes were found in the occipital cortex ($\eta^2=0.530$, t=4.746, p<0.001), parietal cortex ($\eta^2=0.455$, t=4.087, p=0.001) and the posterior cingulate cortex ($\eta^2=0.437$, t=3.941, p=0.001); figure 1. Inter-subject variation was similar in male and female groups. However, females using oral contraceptives tended to have lower mean V_T than females without contraception or recent menopause. Due to low number of subjects in the subgroups no further statistics were attempted; figure 2.

Figure 1. V_T of $[^{18}\text{F}]\text{FMPEP-d}_2$ is higher in males (grey bars, $n=11$) than in females (black bars, $n=11$) in a regionally differential manner. The bars, representing group means in 17 VOIs, are arranged in order of increasing maximum mean V_T . Error bars indicate standard error of mean. Abbreviations: BSTEM, brainstem; THA, thalamus; CER, cerebellum; OCC, occipital cortex; HIPP, hippocampus; CAU, caudate nucleus; PHIPP, parahippocampal gyrus; PAR, parietal cortex; PCC, posterior cingulate cortex; AMY, amygdala; FC, frontal cortex; PFC, prefrontal cortex; INS, insula; OFC, orbitofrontal cortex; TEMP, temporal cortex; ACC, anterior cingulate cortex; PUT, putamen. * $P<0.05$; ** $P<0.01$, repeated measures ANOVA. + $P<0.05$; ++ $P<0.01$, Mann-Whitney U-test.

Figure 2. Scatter plots show higher $[^{18}\text{F}]\text{FMPEP-d}_2$ V_T in males ($n=11$) compared to females ($n=11$) in the occipital cortex (left) and the prefrontal cortex (right). Female subjects using combined oral contraceptives, no contraceptives, or in menopause are denoted with either closed squares ($\blacksquare$, $n=6$), open squares ($\square$, $n=4$), or a diamond ($\blacklozenge$, $n=1$), respectively.

3.2 Parametric image analyses

A voxel-wise t-test showed one large significant cluster of higher V_T in males than females ($kE \geq 250679$, $p_{FDR-corr} < 0.001$); figure 3. Voxel-wise regression analyses revealed two significant clusters ($p_{FDR-corr} < 0.05$) of parametric V_T correlated negatively with visuospatial working memory task (Spatial span) performance. Sex was used as a covariate; figure 4. However, only the left hemisphere cluster survived correcting the cluster extent threshold for multiple comparisons ($p_{FWER-corr} < .008$). There were no gender differences in visuospatial working memory test scores in this sample ($df=20$, $t=-0.537$, $p=0.597$), nor significant associations of other neuropsychological test performance with parametric CB1R V_T .

Figure 3. Statistical parametric mapping (SPM) analysis shows one cluster of higher V_T in males ($n=11$) compared to females ($n=11$). The cluster, visualized here in two horizontal planes representing high T-values, was significant after FDR correction ($p_{FDR-corr} < 0.001$, $kE \geq 250679$). The lower limit of the color bar denotes T-score height threshold and the upper limit denotes maximum peak value (-21mm, -54mm, 14mm).

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Figure 4. SPM analysis shows two clusters where behavioural visuospatial working memory task performance was negatively correlated with V_T . The clusters are shown mapped onto inflated cortical surfaces of the left and right hemispheres separately. See supplement 3 for visualization of the clusters in glass brain. The left cluster was significant after FDR correction ($p_{FDR-corr}=0.001$, $kE \geq 12011$). The right cluster was significant after FDR correction ($p_{FDR-corr}=0.011$, $kE \geq 6887$), but did not survive correction for multiple comparisons ($p_{FWER-corr} < .008$). The lower limit of the color bar denotes T-score height threshold and the upper limit denotes maximum peak value (-36mm, 18mm, 28mm). Symbols mark the cortical area of association (*: middle frontal gyrus and sulcus; **: inferior frontal sulcus and inferior part of precentral sulcus; ▲: central sulcus; ▲▲: intraparietal sulcus and transverse parietal sulci; ▲▲▲: superior frontal gyrus, and anterior cingular gyrus and sulcus; +: superior frontal sulcus; ++: circular sulcus of the insula; +++: anterior cingular gyrus and sulcus).

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4 DISCUSSION

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In this study we found a higher V_T of [^{18}F]FMPEP-d2 in males compared to females. Our results are in good agreement with previous animal studies. The binding of CB1R agonist [^3H]CP55,940 is consistently higher in male rats¹²⁻¹⁴. Also, higher anterior pituitary CB1R mRNA and hippocampal CB1R protein levels have been measured in male rats using in situ hybridization and western blot experiments^{10,11}. Sex differences in ECS responses to stress have also been reported. CB1R agonist [^3H]CP55,940 binding in response to prenatal and postnatal stress is different, with reduced binding in male rats and elevated binding in females¹⁴. Chronic unpredictable stress also results in reduced CB1R protein levels in male adolescent rats, while female levels increase¹⁰. Maternal deprivation stress in turn associates with reduced

immunoreactive CB1R proteins in male rats¹⁹, and subtle increases of [³H]CP55,940 binding in female rats¹³.

While preclinical results largely parallel each other, previous human PET studies on sex difference of brain CB1R are inconsistent. Lower CB1R V_T was reported in males using [¹¹C]OMAR PET^{15,16}, while an opposite finding was reported by another study using standardized uptake values of [¹⁸F]MK-9470 PET¹⁷. The human studies on CB1R sex difference are largely comparable in terms of age, BMI and years of education of healthy control subjects. However, since the ECS interact bidirectionally with sex hormones⁵⁰, inconsistencies between results may be due to differences in hormonal status of female subjects. For example, circulating estradiol levels can vary ten-fold during the normal ovarian cycle⁴⁴. Estrogen significantly affects CB1R agonist binding⁵¹ and increase AEA synthesis in the hypothalamus⁵², while AEA regulates central gonadotropin secretion influencing sex steroid levels⁵². Previous literature on sex difference of endocannabinoid levels in cerebrospinal fluid or peripheral blood is inconclusive with mixed results. Most studies report no major differences of EC levels between males and females^{53–62} but the link between peripheral ECS drive and central CB1 receptor availability is not known⁶³. In our sample, females using combined oral contraceptives, a combination of estrogen and progesterone, tended to have lower mean CB1R availability compared to that of females without hormonal contraception. The inter-subject variation of [¹⁸F]FMPEP-*d2* V_T within the studied areas did not differ between males and females suggesting that the putative effect of combined oral contraceptives, or estrous cycle status, is subordinate to the overall sex effect⁶⁴. However, the effect of contraceptives on CB1R availability cannot be generalized due to cross-sectional study design and a small sample size. It is possible that this association is caused by other circumstances co-occurring with oral contraceptive use. Notably one previous study reported that CB1R binding did not differ between premenopausal females on and off contraceptives¹⁷. The lack of detailed estrous cycle data and used contraceptive characteristics makes it very difficult to assess comparability of studies in this regard^{15–17}.

It is notable that the previous study reporting a lower CB1R availability in males used [¹¹C]OMAR as a PET tracer, which is a rimonabant analogue CB1R antagonist. It is functionally similar to inverse agonist radioligands [¹⁸F]FMPEP-*d2* and [¹⁸F]MK-

9470, which in turn demonstrated higher CB1R availability in males. The latter radioligands have markedly slower kinetics, greater affinities and higher lipophilicities compared to [^{11}C]OMAR. Both [^{18}F]FMPEP-*d2* and [^{11}C]OMAR exhibit specific binding to CB1R that can be displaced with rimonabant. However, this does not necessarily mean that the tracers label exactly same receptor populations. A substantial portion of CB1R are known to undergo receptor internalization processes⁴⁹, the CB1R exists in multiple affinity states in relation to agonist activity⁶⁵, and appears to have many regulatory protein interactions⁶⁶. More specifically, CB1R exist in an active conformation associated with coupling to and activating G-proteins, as well as an inactive conformation^{67,68}. Antagonists/inverse antagonists have equal affinities to the active and inactive states of CB1R, and agonists have preference for the active state. While a substantial majority of CB1R on the neuronal membrane reside in the inactive state, agonists are thought to shift this proportion of active/inactive conformities towards the active state.⁶⁵ It is thus possible that using radiotracers differing in affinity, lipophilicity, receptor-ligand interactions, and scan times may lead to differences in distribution volumes. For example, varying degrees of radioligand displacement by endogenous agonists can lead to differing populations of receptor states and cellular compartment distributions represented by V_T . It is not known whether [^{18}F]FMPEP-*d2* V_T is affected by endogenous agonists, but this would be unlikely considering that [^{11}C]MePPEP, a close structural relative, is not displaced by endogenous agonists or synthetic agonists even at potent levels⁶⁹. This indicates that [^{18}F]FMPEP-*d2* V_T is rather an index of CB1R availability than receptor affinity meaning limited sensitivity to endogenous ligand competition at CB1Rs. To our knowledge no such data is available for [^{11}C]OMAR. Moreover, the proportion of non-displaceable binding to displaceable binding of [^{18}F]FMPEP-*d2* has been previously reported to be small³³, and any non-significant differences in the transport of tracer from blood to tissue compartments are very unlikely to explain any robust differences in V_T .

Opposite results of CB1R V_T using [^{18}F]FMPEP-*d2* and [^{11}C]OMAR have been reported also in studies of alcohol dependence in males. [^{18}F]FMPEP-*d2* V_T remained lower compared to controls after 2-4 weeks of abstinence³⁹, while [^{11}C]OMAR V_T was higher after 4 weeks of alcohol abstinence⁷⁰. Additionally, in a study of CB1R in cannabis dependence, cannabis dependent males had ~20% lower [^{18}F]FMPEP-*d2* V_T ,

which increased to control levels after 4 weeks of abstinence³⁸. Chronic cannabis use similarly resulted in ~15% lower V_T with [^{11}C]OMAR. However, this level increased significantly after only two days of abstinence⁷¹. The initial depression is consistent with CB1R down-regulation secondary to chronic CB1R agonist Δ^9 -THC exposure^{72–74}, but the prompt increase of V_T could also be explained by depletion of cumulated Δ^9 -THC from adipocytes⁷⁵.

Curiously, higher binding of [^3H]OMAR to prefrontal cortex cryo-sections was found to be inversely associated to CB1R protein and mRNA levels in an *ex vivo* sample of schizophrenia patients⁷⁶. This was suggested to reflect alterations in receptor affinity, or trafficking within the neuron. Differences in tracer selectivity balances of CB1R/CB2R binding, inverse association of [^{11}C]OMAR to CB1R agonist activity, or related affinity changes, could also explain the results discussed above. However, these suggestions are highly speculative and cannot be resolved without a cross validation study with [^{11}C]OMAR and [^{18}F]FMPEP-*d*2, or characterization of radioligand displacement *in vivo* by different cannabinoids.

Definitive conclusions about the functional consequences of the observed sex difference cannot be made at this time due to the elusive and highly intricate regulation of CB1R protein expression, trafficking and degradation. One possible explanation is that higher CB1R availability in males could reflect regulation of receptor protein density in the context of lower ECS signaling. This interpretation is supported by previous observations of reduced CB1R V_T secondary to chronic cannabis use³⁸, and reduced CB1R mRNA⁷⁴ and agonist binding⁷² after chronic Δ^9 -THC in adult male rats. Genetic and pharmacological knockout of monoacylglycerol lipase (MAGL), the main degradative enzyme of 2-AG, induces CB1R downregulation and desensitization in the mouse brain⁷⁷, which suggests that the sex difference in CB1R availability may be mediated by 2-AG signaling. However, the regulation of CB1R mRNA and protein is complex, as shown in a cell culture study of acute and chronic endocannabinoid effects⁷³. Here AEA and 2-AG seemed to display functional selectivity for second messenger activation, which has relevance for rates of receptor internalization, subsequent degradation, and replenishment of presynaptic membrane CB1R populations. Since endocannabinoid synthesis pathways are dissociated and differentially regulated^{5,78,79}, it is also possible that the current

findings may be explained by alterations in endogenous cannabinoid agonists such as specific balances of AEA and 2-AG levels. Allosteric CB1R modulators pregnenolone and lipoxin A4 might also contribute to the observed effect⁸⁰.

The CB1R sex difference was regionally specific with statistically significant differences in both cortical and subcortical regions. The largest effect of different CB1R availability in males and females was centered in and around the posterior cingulate area, which contains the retrosplenial cortex (RSC) and parahippocampal cortex (PHC). These areas are suggested to encode the spatial layout and location of specific visual scenes within a larger spatial environment⁸¹. Recently, sex differences in functional association of these areas to scene-selective stimuli were replicated in a large sample of healthy controls⁸². The RSC has been also suggested to be responsible for transformation of information from a self-centered viewpoint to a world-centered spatial framework⁸³, and has also been found to be involved in speech production and comprehension⁸⁴. Although the nature of RSC processing of speech is not known, it is intriguing to speculate the implications of putative dysfunctional spatial encoding of internal speech in the context of auditory hallucinations. Functional connectivity of the RSC to the superior temporal gyrus, an area involved in auditory processing and language comprehension, seems to be increased in schizophrenia⁸⁵. It is thus possible that a lower ECS drive in males could result in a predisposition to aberrant spatial encoding of speech, manifesting as a vulnerability to auditory hallucinations.

We also found negative associations of visuospatial working memory performance to CB1R availability. The associated areas correspond to cortical regions previously suggested to participate in visuospatial working memory, and processing of the attentional priority of memory content^{86,87}. We suggest that ECS tone modulates the balance of excitation and inhibition of cortical primary neurons in a bilateral occipito-parieto-frontal network responsible for sustaining behaviorally relevant visuospatial content in working memory. For example, lower ECS tone could reduce synchronisation of excitatory afferents and cortical activity, via reduced suppression of inhibition in cortical interneurons, resulting in gradual drifting of working memory content from the origin⁸⁸. The observed association parallels the effects of exogenic CB1R agonists on spatial working memory performance^{25,30}. It is possible that, by disrupting the temporal-spatial selectivity of endocannabinoid neurotransmission,

exogenous agonists similarly impair synchronised neural oscillations. On the other hand, an overactive ECS could also result in ineffective working memory through excessive high-pass filtering of perturbing excitatory activity.

It is notable that males and females did not differ in years of received education, level of functioning, or neuropsychological test scores. Thus, the observed association to visuospatial performance could be secondary to a compensated developmental factor. This indicates that the ECS is calibrated during neurodevelopment to provide an optimal behavioral level in the context of other neurotransmitter system states⁸⁹, hormonal influences⁹⁰ and/or environmental influences¹⁹. However, this interpretation is restricted by insufficient statistical power. Subtle sex differences in visuospatial test performance in our sample cannot be entirely ruled out. The ECS may also be involved in other neuropsychological functions with putative sex differences, such as verbal fluency⁹¹.

The ECS affects biologically fundamental brain functions by gating the hypothalamus-pituitary-adrenocortical axis (HPAA) stress response⁹², and striatal dopamine release by local circuit modulation of amygdala and midbrain efferents respectively⁹³. Differences of ECS drive in males and females might thus manifest as different sensitivities to HPAA activity, or striatal dopamine release in response to stress⁹⁴, a transdiagnostic psychiatric risk factor⁹⁵. This theoretical link between sex dependent psychiatric vulnerabilities and the ECS is supported by observations of the intermediary role of the ECS in estradiol effects on addiction⁹⁰ and anxiety behaviour⁹⁶ in female rats. Estradiol also seems to affect hippocampal inhibitory interneuron activity differently in male and females through an ECS dependent mechanism⁹⁷. Whatever the origin, alternate tones of CB1R mediated modulation could nevertheless be manifested as differences in cognitive performance and responses to stress. Studying ECS function separately in male and female psychosis patients or other relevant syndromes are needed to elucidate whether the observed sex differences are relevant in the context of psychiatric vulnerability.

5 CONCLUSIONS

We observed robust gender differences in CB1R availability with large effect sizes. The widespread effect was regionally selective with the most pronounced effects observed in the posterior limbic cortex. Our results also suggest an association between CB1R availability and visuospatial functioning, a domain suggested to have sex differences in the general population. In addition, combined hormonal contraceptives seemed to affect CB1 receptor availability but this should be further investigated with a larger sample size and detailed characterization of hormonal status. Finally, these results implicate that future human studies of ECS function in different neuropsychiatric disorders should closely control the effects of sex and hormonal status.

Acknowledgements. We thank the staff at Turku PET centre for their assistance. This study was supported financially by the EU FP7 programme (METSU, no. 602478, www.metsu.eu) and Turku University Hospital (State research funding, no. P3848). Mattia Veronese was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London.

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