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Kantonen, Tatu; Karjalainen, Tomi; Isojärvi, Janne; Nuutila, Pirjo; Tuisku, Jouni; Rinne, Juha; Hietala, Jarmo; Kaasinen, Valtteri; Kalliokoski, Kari; Scheinin, Harry; Hirvonen, Jussi; Vehtari, Aki; Nummenmaa, Lauri

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Published in:
NeuroImage

DOI:
[10.1016/j.neuroimage.2020.116922](https://doi.org/10.1016/j.neuroimage.2020.116922)

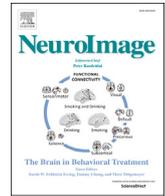
Published: 15/08/2020

Document Version
Publisher's PDF, also known as Version of record

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Please cite the original version:
Kantonen, T., Karjalainen, T., Isojärvi, J., Nuutila, P., Tuisku, J., Rinne, J., Hietala, J., Kaasinen, V., Kalliokoski, K., Scheinin, H., Hirvonen, J., Vehtari, A., & Nummenmaa, L. (2020). Interindividual variability and lateralization of μ -opioid receptors in the human brain: Individual differences in the μ -opioid receptor system. *NeuroImage*, 217, Article 116922. <https://doi.org/10.1016/j.neuroimage.2020.116922>

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Interindividual variability and lateralization of μ -opioid receptors in the human brain

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ARTICLE INFO

Keywords:

μ -Opioid receptors
Endogenous opioids
Positron emission tomography
[¹¹C]carfentanil
Ageing
Brain lateralization

ABSTRACT

Alterations in the brain's μ -opioid receptor (MOR) system have been associated with several neuropsychiatric disorders. Central MOR availability also varies considerably in healthy individuals. Multiple epidemiological factors have been proposed to influence the MOR system, but due to small sample sizes the magnitude of their influence remains inconclusive. We compiled [¹¹C]carfentanil positron emission tomography scans from 204 individuals with no neurologic or psychiatric disorders, and estimated the effects of sex, age, body mass index (BMI) and smoking on [¹¹C]carfentanil binding potential using between-subject regression analysis. We also examined hemispheric differences in MOR availability. Older age was associated with increase in MOR availability in frontotemporal areas but decrease in amygdala, thalamus, and nucleus accumbens. The age-dependent increase was stronger in males. MOR availability was globally lowered in smokers but independent of BMI. Finally, MOR availability was higher in the right versus the left hemisphere. The presently observed variation in MOR availability may explain why some individuals are prone to develop MOR-linked pathological states, such as chronic pain or psychiatric disorders. Lateralized MOR system may reflect hemispheric work specialization in central emotion and pain processes.

1. Introduction

Endogenous opioids modulate multiple physiological and homeostatic functions. The most studied opioid receptors are μ -opioid receptors (MORs), which are widely expressed in the central nervous system (CNS) acting as important mediators for analgesia and reward (Henriksen and Willloch, 2008). Endogenous opioids also regulate mood (Lutz and Kieffer, 2013), social behavior (Machin and Dunbar, 2011) and endocrine function (Katz and Mazer, 2009; Wand et al., 2011). Dysregulation of the MOR system has been documented in multiple disorders and conditions including major depression (Kennedy et al., 2006; Peciña et al., 2019), schizophrenia (Ashok et al., 2019), post-traumatic stress disorder (Liberzon et al., 2007), drug addiction (Contet et al., 2004) and

obesity (Karlsson et al., 2015).

Opioid receptor density varies substantially between healthy humans (Gabilondo et al., 1995; Gross-Isseroff et al., 1990), and this variation may contribute to etiology of different psychiatric conditions as well as treatment responses. First, a polymorphism in the MOR-coding gene OPRM1 influences cerebral MOR availability (Weerts et al., 2013). Second, one positron emission tomography (PET) study suggests that age and sex explain some of this variation: MOR availability increases with advancing age in cortical areas, whereas women have higher MOR availability compared to men during the reproductive years (Zubieta et al., 1999). Third, MOR system dysfunction has been associated with health-related conditions and behavior: Morbidly obese individuals have globally downregulated MORs (Karlsson et al., 2015). Also smoking has

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been associated with altered MOR availability, although the evidence is mixed (Ray et al., 2011; Scott et al., 2007; Weerts et al., 2014). Interpretation and generalization of these findings is problematic due to small sample sizes (typically 20–40) in neuroreceptor PET studies.

Emotion and affective pain processes have been proposed to exhibit hemispheric lateralization in human brain, especially in amygdala (Ji and Neugebauer, 2009; Lanteaume et al., 2007). Since endogenous opioids are fundamentally involved in these processes (Corder et al., 2018; Nummenmaa and Tuominen, 2018), one potential mechanism for functional lateralization could be hemispheric difference in MOR density. Indeed, a study examining post-mortem human brain samples found that some opioid peptides are partly lateralized between hemispheres (Watanabe et al., 2015). Whether MOR density is lateralized *in vivo* is currently not known.

Poor replicability of neuroimaging findings has recently raised significant concerns (Poldrack et al., 2017). Small sample size (Button et al., 2013), ubiquitous ‘researcher degrees of freedom’ (Simmons et al., 2011), inappropriate correction for multiple comparisons (Eklund et al., 2016), and uncertain measurements (Loken and Gelman, 2017) have been highlighted as underlying reasons. Larger samples (Cremers et al., 2017; Poldrack et al., 2017) and stricter statistical thresholds (Benjamin et al., 2018) have been proposed to alleviate the problem. It has also been argued that Bayesian hierarchical modeling could facilitate reproducible research by limiting the number of paths a researcher can take in their analyses (Lindquist and Gelman, 2009) and by removing the need to correct for multiple comparisons (Gelman et al., 2012).

Here we used Bayesian hierarchical modeling with varying slopes and intercepts for different brain regions to estimate the effects of sex, age, body mass index (BMI) and smoking on cerebral MOR availability, and cerebral lateralization of the MOR system in a large sample of 204 historical subjects scanned with PET using [¹¹C]carfentanil, a highly selective MOR-agonist tracer.

2. Materials and methods

2.1. Subjects

The data were retrieved from the AIVO database (<http://aivo.utu.fi>) of *in vivo* molecular images hosted by Turku PET Centre. We identified all the individuals without neurologic or psychiatric disorders who had been scanned with [¹¹C]carfentanil PET between 2003 and 2018 in baseline condition. The resulting data from 204 individuals consists of scans from 11 research projects and five PET scanners. No subjects abused alcohol or illicit drugs or used medications affecting the CNS. 13 females were smokers, while no males smoked. The characteristics of the subjects are summarized in Table 1. Information about the PET scanners, smoking status and handedness is summarized in Supplementary Table 1. The study was conducted in accordance with the Declaration of Helsinki. Because our study was a register-based and retrospective study of historical data, informed consent was waived. The Turku University Hospital Clinical Research Services approved the study.

2.2. Image processing and binding quantification

Preprocessing of the PET data as well as its kinetic modeling were done using Magia (Karjalainen et al., 2020) (<https://github.com/tkjarjal/magia>), an automated processing pipeline developed at the Turku PET Centre running on MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States). The preprocessing consisted of framewise realignment and coregistration of the PET and magnetic resonance images (MRIs). Tracer binding was quantified using the outcome measure binding potential (BP_{ND}), which is the ratio of specific binding to non-displaceable binding in tissue (Innis et al., 2007). BP_{ND} was estimated using simplified reference tissue model (Lammertsma and Hume, 1996), with occipital cortex serving as the reference region (Frost et al., 1989).

BP_{ND} is the product of i) density of the target receptors unoccupied by endogenous ligands and ii) affinity of the radioligand to the receptor (Henriksen and Willoch, 2008; Mintun et al., 1984), and it is impossible to differentiate these components with a single PET scan. [¹¹C]carfentanil is an agonist tracer preferably binding in MORs in the high-affinity state (Henriksen and Willoch, 2008). While endogenous opioids compete for binding sites with [¹¹C]carfentanil (Quelch et al., 2014; Saanijoki et al., 2018), at least in rats the basal opioid concentrations are low (Maidment et al., 1989). Thus, [¹¹C]carfentanil BP_{ND} in baseline condition is likely proportional to MOR density, but the exact contributions of MOR density, receptor affinity, and baseline occupancy by endogenous opioids cannot be assessed in a single measurement.

Regions of interest (ROIs) and reference regions were parcellated for each subject using FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/>). The bilateral ROIs consisted of MOR-rich regions: amygdala, caudate, dorsal anterior cingulate cortex, inferior temporal gyrus, insula, middle temporal gyrus, nucleus accumbens, orbitofrontal cortex, pars opercularis, posterior cingulate cortex, putamen, rostral anterior cingulate cortex, superior frontal gyrus, temporal pole, and thalamus. Parametric BP_{ND} images were also calculated for full-volume analysis. They were spatially normalized to MNI-space via segmentation of T1-weighted MRIs and smoothed with an 8-mm Gaussian kernel.

2.3. Statistical analysis

2.3.1. Model specification and estimation

Bayesian hierarchical modeling was used to estimate the effects. The models were specified using the R package BRMS (Bürkner, 2017) that uses the efficient Markov chain Monte Carlo sampling tools of RStan (<https://mc-stan.org/users/interfaces/rstan>). We used weakly informative priors: For intercepts, we used the default of BRMS, i.e. Student’s t-distribution with scale 3 and 10 degrees of freedom. For predictors, a Gaussian distribution with standard deviation of 1 was used to provide weak regularization. The BRMS default prior half Student’s t-distribution with 3 degrees of freedom was used for standard deviations of group-level effects; BRMS automatically selects the scale parameter to improve convergence and sampling efficiency. The BRMS default prior LKJ(1) was used for correlations of group-level random effects.

The ROI-level models were estimated using three chains, each of which had 2000 warmup samples and 10000 post-warmup samples, thus totaling 30000 post-warmup samples. The sampling parameters were slightly modified to facilitate convergence ($adapt_delta = 0.95$; $max_treedepth = 15$). The sampling produced no divergent iterations and the Rhats were all 1.0, suggesting that the chains converged successfully. Before model estimation, continuous predictors were standardized to have zero mean and unit variance, thus making the regression coefficients comparable across the predictors. The scripts used for analyzing the data are available in <https://github.com/tkjarjal/mor-variability>.

2.3.2. Model comparison

We specified seven linear models with varying slopes and intercepts for the ROIs (Supplementary Figure 1). All models included subject-specific

Table 1
Characteristics of the study sample.

	Males (n = 132)			Females (n = 72)		
	mean	SD	range	mean	SD	range
Age (years)	27.8	8.0	20–58	40.7	10.3	20–59
Weight (kg)	78.0	9.3	53–110	76.5	23.5	47–144
Height (cm)	180.7	5.9	165–198	165.8	5.7	151–180
BMI (kg/m ²)	23.9	2.7	18–34	27.8	8.1	18–49
Injected activity (MBq)	297.2	93.5	218–533	341.1	109.0	199–519
Injected mass (μg)	0.46	0.48	0.05–3.80	0.47	0.50	0.08–2.00

intercepts to control for variables not explicitly included in the models and dummy-covariates to control for biases between different scanners. The first model included all variables of interest (sex, age, BMI, and smoking) and sex-interactions for age and BMI; sex-smoking interaction could not be estimated because all smokers were female. The remaining models were submodels of the first model. Binding potentials were log-transformed because posterior predictive checking (Gabry et al., 2019; Gelman et al., 2013) indicated that log-transformation significantly improves model fit (see Supplementary Figure 2). The log-transformation essentially switches the model from additive to multiplicative; it also helps in model fitting because the assumption of linear additivity works poorly when the dependent variable is restricted to positive values (Gelman and Hill, 2006).

Predictive performance of the seven models was compared using Bayesian 10-fold cross-validation (Vehtari and Lampinen, 2002), as implemented in the R package LOO (<https://CRAN.R-project.org/package=loo>). The subsamples used in cross-validation were created by randomly removing 10% of the subjects. Predictive accuracy was then assessed for the leftover subjects. This procedure was repeated ten times, and the results were combined to select a model. According to the cross-validation criterion, the model without BMI (Model 4) had the best predictive accuracy, outperforming the other models significantly (Supplementary Table 2). BMI was thus not a relevant predictor of MOR availability. The posterior distribution obtained using the Model 4 (without BMI) is more closely investigated in the Results section.

2.3.3. Full-volume analyses

Traditional voxel-level analysis with Bayesian hierarchical modeling was inappropriate for the whole-brain analysis: With typically used voxel sizes (isotropic 1–6 mm) the number of voxels was so large that model estimation was computationally prohibitive. In turn, when down-sampling the data to resolution whose analysis was computationally feasible (isotropic 10 mm), the resulting voxels extended over functionally heterogeneous tissue causing partial volume effects. We thus developed a new method for receptor-density-based subdivision of anatomical regions. The method clusters atlas-derived anatomical ROIs into smaller volumetric units based on the spatial receptor-density distribution within each ROI. Here the anatomical ROIs were defined using the AAL template, and the clustering was based on a high-resolution population [^{11}C]carfentanil BP_{ND} map (i.e. average of all parametric BP_{ND} images analyzed in the study; see Fig. 1). Hierarchical clustering, as implemented in MATLAB, was used (see <https://github.com/tkkarja/mor-variability> for the code). The procedure makes the receptor availability of the clusters homogenous within clearly defined anatomical boundaries while keeping the number of volumetric units within computationally feasible limits (here 320). Once the clusters were

defined, we calculated cluster-specific binding potentials for each subject, and fitted the Model 4 to the data, thus essentially estimating the effects in the whole brain.

2.3.4. Hemispheric asymmetry

Hemispheric asymmetry was analyzed by comparing within-subject differences in binding potentials between the hemispheres. We first calculated the difference between the ROI-specific right and left hemisphere estimates. We then modeled these differences using ROI-specific intercepts using the default priors of BRMS. The effects were calculated separately for males and females.

3. Results

In both sexes, MORs were widely expressed in the brain (Fig. 1), consistent with previous studies (Nummenmaa and Tuominen, 2018). Unthresholded atlases of average [^{11}C]carfentanil BP_{ND} for males, females and the whole sample, and beta maps for age-effect in females, age-effect in males, and the smoking effect are available in <https://neurovault.org/collections/GCELSAIA/>.

3.1. Effects of age and sex

We observed regionally varying effects of age on [^{11}C]carfentanil BP_{ND} across both sexes (Fig. 2). BP_{ND} decreased with age in amygdala, thalamus, nucleus accumbens, and cerebellum, whereas it increased with age in temporal regions and frontal cortex. The positive associations were stronger in males. In males, [^{11}C]carfentanil BP_{ND} increased with age also subcortically in putamen and insula. The proportional changes of BP_{ND} as a result of 10.8-year increase in age (one SD of the sample's age distribution) in the ROIs are presented in Supplementary Table 3 – these frontotemporal increases ranged from 3 to 16% and subcortical decreases from 2 to 8%. Age-dependent sex-differences in [^{11}C]carfentanil BP_{ND} are visualized in Fig. 3. In almost all ROIs, the mean BP_{ND} was higher in 20-year-old females compared to 20-year-old males (Supplementary Table 3). Because BP_{ND} increased with age in males faster than in females, the sex-differences decreased until around 30 years of age, after which BP_{ND} in males increased above females in most brain regions. Amygdala, thalamus, nucleus accumbens, and temporal pole displayed no notable sex differences at any age.

We also explored nonlinear effects of age on [^{11}C]carfentanil BP_{ND} in the ROIs (Supplementary Figure 3). These analyses confirmed that BP_{ND} decreases linearly in amygdala and thalamus, and the increase in BP_{ND} is steeper in males compared to females in most brain regions. In males, however, the increase was linear only until approximately 30 years of

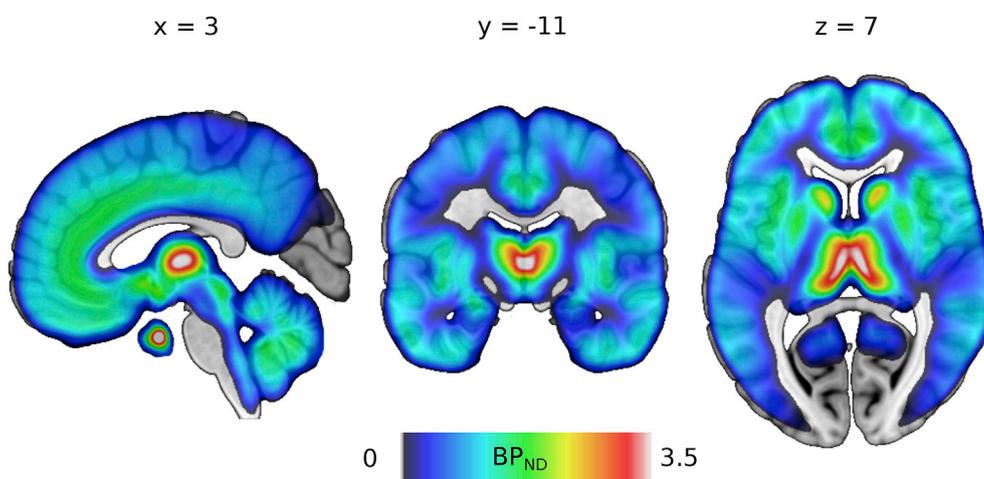


Fig. 1. Mean distribution of μ -opioid receptors in the human brain based on the 204 [^{11}C]carfentanil BP_{ND} images.

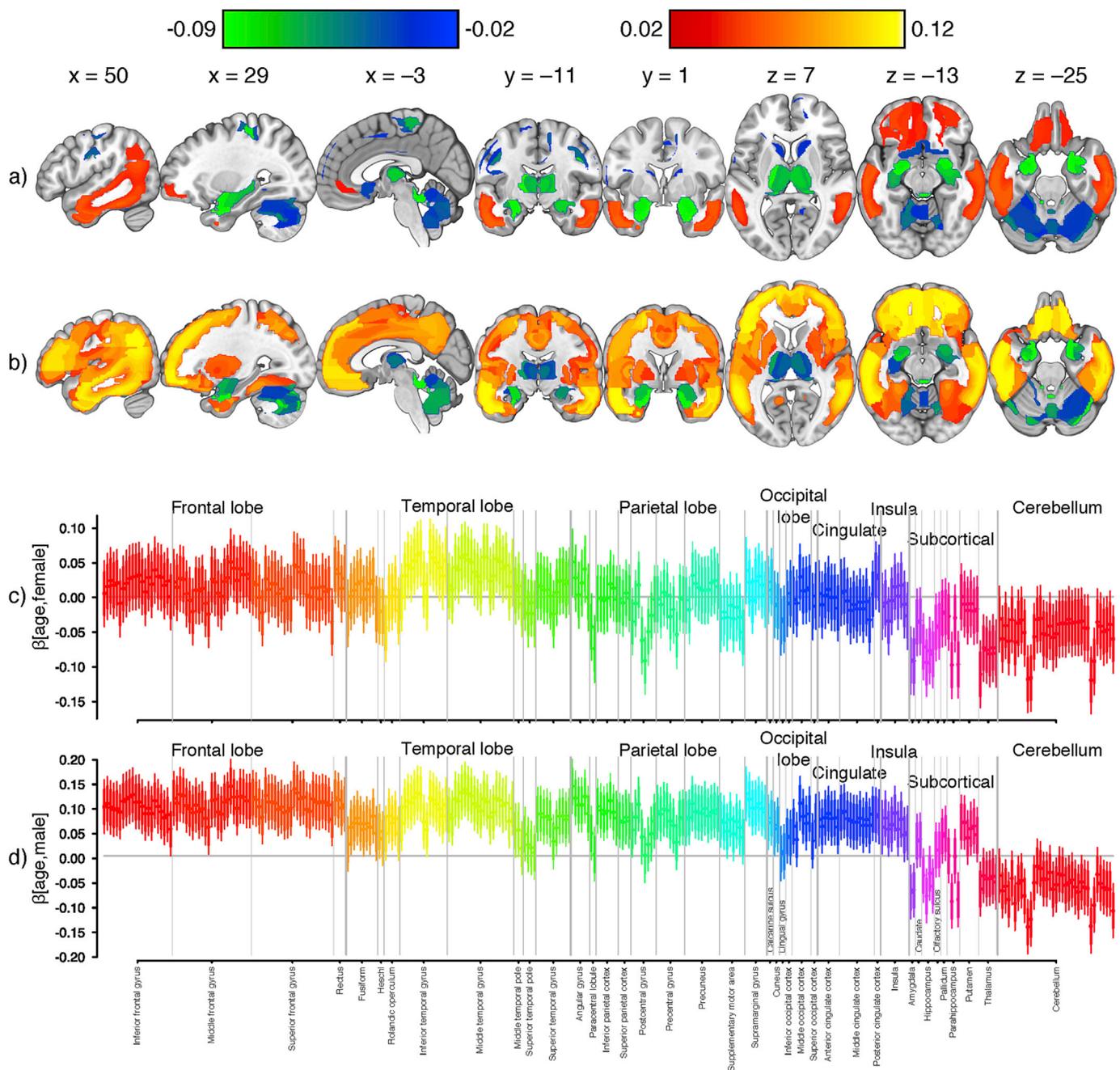


Fig. 2. Effects of age and sex on μ -opioid receptor (MOR) availability in the brain. (a–b) Brain regions where $[^{11}\text{C}]$ carfentanil BP_{ND} is associated with age in females (a) and males (b). Shown clusters have 80% posterior interval excluding zero and absolute value of the regression coefficient at least 0.02. Coloring indicates increase (hot colors) and decrease (cool colors) of BP_{ND} as a function of age. Unthresholded maps are available at <https://neurovault.org/collections/GCELSAIA/>. (c–d) Summary of the posterior distributions of all clusters for the regression coefficient of age for females (c) and males (d). The filled circles represent posterior means, the thick lines 80% posterior intervals, and the thin lines 95% posterior intervals. Color coding is used for highlighting the anatomical grouping of the clusters (see labels on x-axis).

age, after which it slowed down and saturated by 50 years of age. In temporal and frontal cortices, also females exhibited similar nonlinear age-effects.

3.2. Effects of smoking

Smokers had globally decreased binding compared to nonsmokers (Supplementary Figure 4). The reductions, ranging from 8% to 14% (Supplementary Table 3), were most prominent in subcortical regions such as amygdala and striatum. Because all the smokers were female, we also estimated the effects of smoking using female-only data. This

analysis yielded similar results.

3.3. Hemispheric asymmetry

The posterior distributions for absolute differences between regional binding potentials in the right and left hemispheres are presented in Fig. 4. All differences were within $\pm 6\%$ (Supplementary Table 4). In most brain regions, including thalamus, caudate, cingulate cortex, orbitofrontal cortex, and putamen, $[^{11}\text{C}]$ carfentanil BP_{ND} was higher in the right hemisphere. Only in nucleus accumbens and amygdala was BP_{ND} higher in the left hemisphere. This lateralization was consistent across

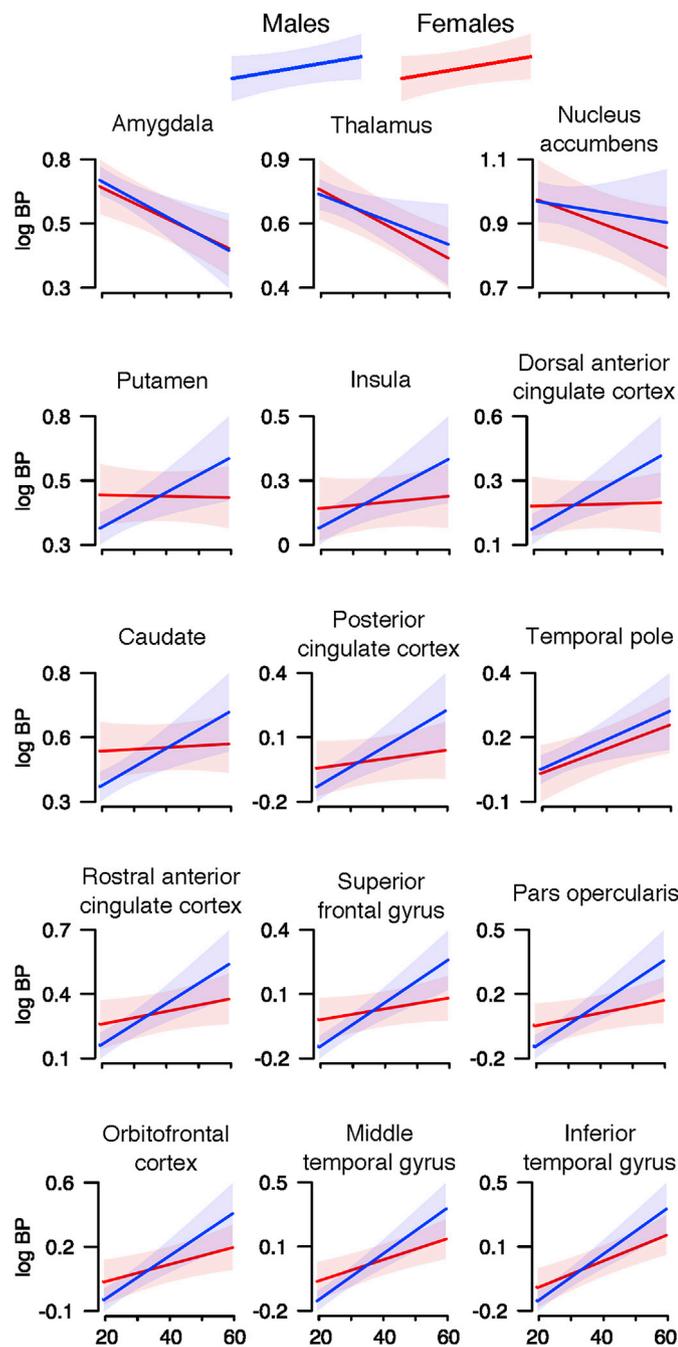


Fig. 3. Sex-dependent age effects. Age-dependent mean log binding potential along with 95% highest density posterior intervals calculated over the range of data points for all regions of interest separately for males (blue) and females (red).

the sexes. We also examined hemispheric differences in subsamples of the 128 right-handed and the seven left-handed individuals, and found that both groups display lateralization towards the right hemisphere.

4. Discussion

Our main findings were that sex, age, and smoking influence [¹¹C]carfentanil binding in the brain. BMI was not associated with [¹¹C]carfentanil BP_{ND} . In most brain regions, BP_{ND} was slightly higher in the right than in the left hemisphere.

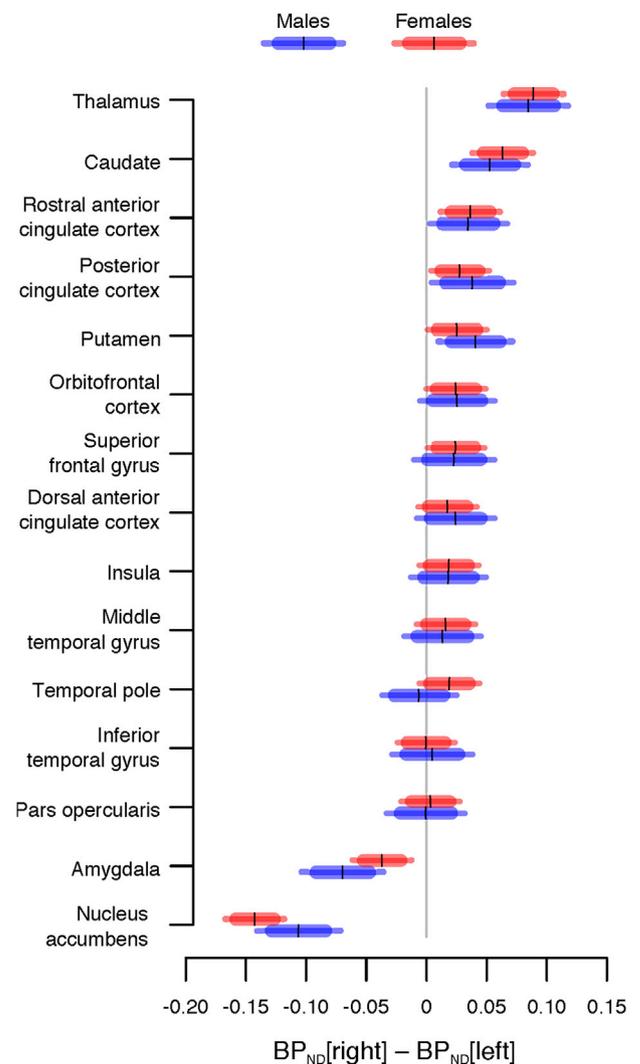


Fig. 4. Posterior distribution of hemispheric differences estimated in each region separately for females (red) and males (blue). Distributions located right to the dashed line have higher binding in the right hemisphere and vice versa. The black vertical lines represent posterior means, the thick horizontal bars 80% posterior intervals, and the thin horizontal bars 95% posterior intervals.

4.1. Effects of age

Older age was associated with reductions in [¹¹C]carfentanil BP_{ND} in thalamus, amygdala, nucleus accumbens, and cerebellum. On the contrary, BP_{ND} in frontotemporal areas increased with age in both sexes. Nonlinear models revealed that this upregulation reached a plateau at around 40–50 years of age. The age-related increase in cortical MOR availability accords with prior autoradiography studies (Gabilondo et al., 1995; Gross-Isseroff et al., 1990; Zalsman et al., 2005). The only, significantly smaller, prior PET study investigating ageing and MOR reported a positive association in cortical areas (Zubieta et al., 1999). Our data thus confirm this cortical increase of MOR availability with advancing age, but highlight that the increase saturates at midlife and that the effects of age are bidirectional and region-specific.

Our cross-sectional study cannot point an exact reason for detected age-related decrease in [¹¹C]carfentanil BP_{ND} in thalamus, amygdala and nucleus accumbens, but it is possible that it could be at least partly caused by brain atrophy. Ageing is associated with gray matter (GM) loss in the brain (Good et al., 2001; Lockhart and DeCarli, 2014), and many other receptor systems, including serotonin and dopamine, undergo receptor

loss with advancing age (Karrer et al., 2017; Rodriguez et al., 2012). Another possibility is that the decrease in BP_{ND} reflects age-associated changes in central pain processes. In PET studies, fibromyalgia and central neuropathic pain are associated with reduced opioid receptor availability in nucleus accumbens, amygdala and thalamus (Harris et al., 2007; Jones et al., 2004). These brain regions also display enhanced opioidergic processing during acute pain (Zubieta et al., 2001). In epidemiological studies, older age is associated with higher prevalence of musculoskeletal conditions (Woolf and Pfleger, 2003) and chronic pain (van Hecke et al., 2013). On the other hand, older adults have decreased sensitivity to low-intensity pain (Lautenbacher et al., 2017).

While consistent with prior findings, the age-related increase in cortical [^{11}C]carfentanil BP_{ND} is in stark contrast with age-associated loss of GM density. Peculiarly, the increase in BP_{ND} likely reflects actual increase in receptor density, as data from rodents indicate that MOR density increases with age (Petrillo et al., 1987), and human autoradiography studies suggest that the age-related increase in cortical MOR availability results from increased receptor density rather than affinity (Gabilondo et al., 1995; Gross-Isseroff et al., 1990). As ageing decreases endogenous opioid concentration in rodent CNS (Barden et al., 1981), it is possible that the receptors are upregulated to compensate for the decreased endogenous opioid drive (Lesscher et al., 2003).

4.2. Sex differences

Effects of age on [^{11}C]carfentanil BP_{ND} were stronger in males, and also observed more widely in the brain than in females. One previous study suggests that during their reproductive years females have higher MOR availability than males in thalamus, caudate, and amygdala, but that the difference disappears or reverses after menopause (Zubieta et al., 1999). Another study with age-matched 40-year-old participants found higher MOR availability in males in cingulate cortex and ventral striatum (Weerts et al., 2011). The present large-scale dataset suggests that the sex difference in MOR availability is actually reversed during ageing: In the reproductive age women have higher MOR availability than men in multiple brain regions, whereas older males have higher MOR availability in cortical and most subcortical areas. These sex differences might be linked with gonadal steroids: In rodents, subcutaneous injection of estrogen increases MOR mRNA levels in the CNS (Quiñones-Jenab et al., 1997). Human studies have shown that in females the estrogen-state influences MOR availability in thalamus, amygdala and nucleus accumbens (Smith et al., 2006). Menopause and the associated low-estrogen state in older females might thus explain some of the observed sex differences. Also in males, the estrogen-to-testosterone ratio increases with age (Cooke et al., 2017), and the elevated estrogen tone might in turn upregulate MORs.

In some brain regions, the age-dependencies were nonlinear. In males, [^{11}C]carfentanil BP_{ND} increased rapidly between the ages 20 and 30, after which the increase slowed down and plateaued at around 40–50 years. Similar saturation pattern was observed in females in frontal and temporal cortices. These data indicate that the linear sex-interactions that were observed in temporal cortex could result from differences in the age distributions rather than from biological sex differences. Elsewhere in the brain, the steeper increase of BP_{ND} in males is more likely to be of biological origin.

4.3. Lateralization of μ -opioid receptors

[^{11}C]carfentanil BP_{ND} was 1–5% higher in the right than in the left hemisphere; only in nucleus accumbens and amygdala BP_{ND} was lateralized to the left hemisphere. A post-mortem study found lateralization of MOR agonists in human cingulate cortex (Watanabe et al., 2015), showing that opioid peptide concentrations may differ between the hemispheres, possibly explaining some of the lateralization.

Previous studies have revealed functional asymmetry in amygdala's emotion processing. Electrical stimulation of right amygdala produces

only negative emotions, whereas stimulation of left amygdala induces both positive and negative emotions (Lanteaume et al., 2007). In PET studies, depression associates with lowered MOR availability in amygdala (Kennedy et al., 2006), while positive mood leads to MOR activation in amygdala (Koepp et al., 2009). Amygdala has excitatory effects on ipsilateral nucleus accumbens, facilitating motivated behavior (Stuber et al., 2011). Nucleus accumbens and amygdala were the only regions where the MOR system was left-lateralized. MOR lateralization in amygdala and nucleus accumbens might reflect lateralization of emotion processing, the left amygdala with higher MOR availability being more responsible for emotional processes.

Thalamus showed the most prominent rightward lateralization. Thalamus relays somatosensory and nociceptive signals to cerebral cortex (Herrero et al., 2002). Acute pain is associated with endogenous opioid release in thalamus, presumably to alleviate pain (Zubieta et al., 2001). Right thalamus may be more specialized to pain processing than the left, since following a stroke and brain damage, thalamic pain syndrome develops more frequently if the thalamic lesion has been right-sided (Nasreddine and Saver, 1997). Our finding of increased MOR availability in the right thalamus is consistent with lateralized central pain processing.

4.4. Effects of smoking and BMI

Smokers had globally decreased [^{11}C]carfentanil BP_{ND} in striatum, insula and amygdala. This is in line with prior studies (Scott et al., 2007; Weerts et al., 2014), but our cross-sectional study cannot establish a causal mechanism between smoking and MORs. Nicotine administration induces endogenous opioid release (Davenport et al., 1990), a mechanism that presumably underlies nicotine-induced reward (Walters et al., 2005). Regular smoking thus frequently activates the endogenous opioid system, and our data suggest that it might lead to downregulation of MORs, possibly to prevent overactivation of the MOR system.

Against our expectations BMI was not associated with [^{11}C]carfentanil BP_{ND} even though MOR availability is decreased in obesity (Burghardt et al., 2015; Karlsson et al., 2015) and binge eating disorder (BED) (Majuri et al., 2017). Because our subjects were mostly normal-weight, BMI may be associated with MORs only in the pathological (obese) end of the spectrum. BMI is widely used as a marker for obesity and excess fat, but it does not directly measure body composition, and it does not necessarily reflect the changes of body adiposity arising from factors such as subject's age or muscle mass (Prentice and Jebb, 2001). Lowered MOR availability in the morbidly obese and BED patients may therefore result from pathological eating or dysfunction of the pleasure system rather than from high body mass itself.

4.5. Limitations

Our data were sampled from 11 distinct projects using five PET scanners. Although the scanners are cross-calibrated, they may produce slightly different BP_{ND} estimates. We however corrected for potential scanner-related biases in the analyses. The data were not ideally detailed – for example, in females, age of the possible menopause or the phase of menstrual cycle was not recorded. We had subjects with 20–60 years of age, and the conclusions may not be generalizable outside this range. The data was not fully representative for all subgroups – there were only seven confirmed left-handed subjects and 13 smokers, and all smokers were females. Genetic data were not available for the historical subjects – future studies could examine how the OPRM1 A118G polymorphism influences the expression and lateralization of MORs.

5. Conclusions

Age, sex, and smoking influence cerebral MOR availability. Older age was associated with decreased MOR availability in thalamus and amygdala, possibly reflecting age-associated neurodegeneration or pain

processes. In frontotemporal regions, MOR availability increased with age, which might reflect a compensatory process to reduced endogenous opioid concentrations. MOR availability increases with age faster in males than in females, and this may pertain to sex-specific hormonal modulation of MOR system. Smoking was associated with decreased MOR availability, possibly resulting from receptor downregulation as a consequence of continuous activation of MORs. BMI was however not associated with MOR availability. Finally, MOR system manifests consistent but minor hemispheric asymmetry. From experimental point of view, these data highlight that studies with [¹¹C]carfentanil need to have well-matched, non-smoking samples and single-sex subject selection, unless sufficient statistical power for analysing sex differences can be achieved. Given the prominent effect of age on MORs especially before the age of 30, even small age differences between tested groups can lead to artificial group differences. Altogether, these data show that healthy humans vary significantly in their cerebral MOR availability, and that age, sex and smoking status explain a part of this variation. Variability in MOR system might be one neurobiological mechanism explaining why some individuals are more vulnerable to develop MOR-linked pathological states such as chronic pain or neuropsychiatric disorders.

Funding, disclosure and acknowledgements

This study was supported by Academy of Finland (grant #294897# to L.N.) and Sigrid Juselius Foundation. We thank the Finnish Cultural Foundation (Southwest Finland Fund) for personal grants to T. Kantonen and T. Karjalainen, as well as State Funding for University-level Health Research given to T. Karjalainen. We also thank the Emil Aaltonen Foundation for personal grant to T. Kantonen. Declarations of interest: none. We would like to acknowledge Semi Helin for his contribution to the reporting of [¹¹C]carfentanil radiochemical data. Earlier version of this manuscript has been posted on preprint server bioRxiv: <https://www.biorxiv.org/content/10.1101/821223v1>.

Data and code availability statement

Preprocessing of the PET data as well as its kinetic modeling were done using *Magia*, and the code is available in <https://github.com/tkkarjal/magia>. The scripts used for analyzing the data are available in <http://github.com/tkkarjal/mor-variability>. Unthresholded atlases of average [¹¹C]carfentanil *BP_{ND}* for males, females and the whole sample, and beta maps for age-effect in females, age-effect in males, and the smoking effect are available in <https://neurovault.org/collections/GCELSAIA/>.

CRediT authorship contribution statement

Tatu Kantonen: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Tommi Karjalainen:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing - review & editing, Visualization, Funding acquisition. **Janne Isojärvi:** Software, Resources, Data curation, Writing - review & editing. **Pirjo Nuutila:** Investigation, Writing - review & editing. **Jouni Tuisku:** Methodology, Writing - review & editing. **Juha Rinne:** Investigation, Writing - review & editing. **Jarmo Hietala:** Investigation, Writing - review & editing. **Valtteri Kaasinen:** Investigation, Writing - review & editing. **Kari Kalliokoski:** Investigation, Writing - review & editing. **Harry Scheinin:** Investigation, Writing - review & editing. **Jussi Hirvonen:** Investigation, Writing - review & editing. **Aki Vehtari:** Methodology, Software, Validation, Resources, Writing - review & editing. **Lauri Nummenmaa:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing - review & editing, Project administration, Funding acquisition, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neuroimage.2020.116922>.

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