Published in final edited form as:

Neuroimage. 2013 August 15; 77: . doi:10.1016/j.neuroimage.2013.03.027.

5-HT_{1A} Sex Based Differences in B_{max}, in vivo K_D, and BP_{ND} in the Nonhuman Primate

Dustin W. Wooten, M.S.^{1,2}, Ansel T. Hillmer, M.S.^{1,2}, Jeffrey M. Moirano, M.S.^{1,2}, Dana L. Tudorascu, Ph.D.³, Elizabeth O. Ahlers, B.S.², Maxim S. Slesarev, B.S.², Todd E. Barnhart, Ph.D.¹, Jogeshwar Mukherjee⁵, Mary L. Schneider, Ph.D.^{2,4}, and Bradley T. Christian, Ph.D.^{1,2,6}

¹Department of Medical Physics, University of Wisconsin-Madison, Madison, WI 53705, USA

²Waisman Laboratory for Brain Imaging and Behavior, University of Wisconsin-Madison, Madison, WI 53705, USA

³Division of General Internal Medicine, University of Pittsburgh, Pittsburgh, PA 15260, USA

⁴Department of Kinesiology, University of Wisconsin-Madison, Madison, WI 53705, USA

⁵Department of Radiological Sciences, University of California-Irvine, Irvine, California 92697, USA

⁶Department of Psychiatry, University of Wisconsin-Madison, Madison, WI 53705, USA

Abstract

Serotonin (5-HT) dysfunction has been implicated in neuropsychiatric illnesses and may play a pivotal role in the differential prevalence of depression between the sexes. Previous PET studies have revealed sex-based differences in 5-HT_{1A} binding potential (BP_{ND}). The binding potential is a function of the radioligand-receptor affinity (1/K_{Dapp}), and receptor density (B_{max}). In this work, we use a multiple-injection (MI) PET protocol and the 5-HT_{1A} receptor antagonist, [¹⁸F]mefway, to compare sex-based differences of in vivo affinity, B_{max}, and BP_{ND} in rhesus monkeys.

Methods—PET [¹⁸F]mefway studies were performed on 17 (6m, 11f) rhesus monkeys using a 3-injection protocol that included partial saturation injections of mefway. Compartmental modeling was performed using a model to account for non-tracer doses of mefway for the estimation of K_{Dapp} and B_{max}. BP_{ND} estimates were also acquired from the first injection (high specific activity [¹⁸F]mefway, 90 minute duration) for comparison using the cerebellum (CB) as a reference region. Regions of interest were selected in 5-HT_{1A} binding regions of the hippocampus (Hp), dorsal anterior cingulate cortex (dACC), amygdala (Am), and raphe nuclei (RN).

Results—Female subjects displayed significantly (*p<0.05) lower K_{Dapp} in the Hp (−32%), Am (−38%), and RN (−37%). Only the Hp displayed significant differences in B_{max} with females having a B_{max} of −29% compared to males. Male subjects demonstrated significantly lower BP_{ND} measurements in the Am (14%) and RN (29%).

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Corresponding Author: Dustin W. Wooten, M.S. Department of Medical Physics University of Wisconsin-Madison 1111 Highland Avenue, Madison, WI 53705 dwooten@wisc.edu Telephone: (608) 262-2170 Fax: (608) 262-2413.

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Conclusion—These results suggest that the higher BP_{ND} values found in females are the result of lower [^{18}F]mefway K_{Dapp} . Although a more experimentally complex measurement, separate assay of K_{Dapp} and B_{max} provides a more sensitive measure than BP_{ND} to identify the underlying differences between females and males in 5-HT $_{1A}$ function.

Keywords

5-HT; serotonin; 5-HT $_{1A}$; B_{max} ; K_D ; Sex Differences

1 Introduction

Major differences in serotonin (5-HT) function between males and females are believed to play a role in the higher prevalence of depression among females (Cahill et al, 2006; Cosgrove et al, 2007). The biological bases for these sex-specific functional differences in the 5-HT system remain unclear, however, could be attributed to gonadal hormones that affect serotonin levels. Variations in sex-dependent 5-HT function have been shown for blood serotonin levels (Ortiz et al, 1988), serotonin synthesis rates (Nishizawa et al, 1997; Sakai et al, 2006), serotonin transporter binding levels, and 5-HT neuroreceptor-ligand binding (Biver et al, 1996; Parsey et al, 2002; Jovanovic et al, 2008; Maron et al, 2010; Meltzer et al, 2001). A critical regulator of the 5-HT system, 5-HT $_{1A}$ receptors have been a central focus of research in sex-specific function. In vitro studies examining sex-based differences in the 5-HT $_{1A}$ system have been inconclusive. Using [^3H]8-OH-DPAT, a 5-HT $_{1A}$ agonist, in vitro binding assays showed similar measures of receptor density (B_{max}) and equilibrium dissociation (K_D) between the sexes (Dillon et al., 1991; Matsubara et al., 1991). Postmortem studies comparing receptor densities in suicide victims and controls showed higher [^3H]8-OH-DPAT binding in females (Arango et al, 1995). The effects of age on the 5-HT $_{1A}$ system have been reported to alter B_{max} in the parietal cortex, occipital cortex, and hippocampus of females and K_{Dapp} in the occipital cortex of males (Palego et al., 1997). Positron emission tomography (PET) imaging may provide an opportunity to more closely characterize in vivo sex-specific differences in B_{max} and K_D .

The binding metric frequently used for PET neuroreceptor studies is the nondisplaceable binding potential, BP_{ND} (Innis et al, 2007), representing the composite parameter of $f_{ND}B_{avail}/K_D$. In addition to serving as an index of B_{max} , the BP_{ND} ratio can be influenced by the receptor affinity state and endogenous neurotransmitter competition at the receptor site via K_{Dapp} . The detection of concomitant changes in these measures may be masked in the BP_{ND} metric. The use of a multiple injection (MI) method, which includes the administration of partially saturating ligand, allows the separate identification of the binding parameters B_{max} and K_{Dapp} . Within this experimental context, effects of endogenous 5-HT competition are inherent to the in vivo equilibrium dissociation rate constant, $K_{Dapp}(=k_{off}/k_{on})$, via the association rate constant, k_{on} . It is this additional detail of 5-HT $_{1A}$ function, beyond the BP_{ND} measurement, that will assist in clarifying the differences in the 5-HT system between the sexes that may ultimately provide insight into 5-HT disruption present in neuropsychiatric illnesses such as depression.

In this work, we use the MI technique with the 5-HT $_{1A}$ receptor antagonist [^{18}F]mefway to uncouple the binding parameters K_{Dapp} and B_{max} . The MI technique is a nonequilibrium approach that uses multiple injections of radioligand of varying specific activity in a single scanning duration. Our injection scheme restricts the first injection to high specific activity [^{18}F]mefway over a 90 minute duration allowing estimation of reference region based BP_{ND} . Our previous validation studies involved the use of [^{18}F]mefway MI PET to measure B_{max} across the regions of the brain (Wooten et al, 2012). To enhance parameter identifiability in the brain regions with low 5-HT $_{1A}$ receptor binding, the assumption of a

constant K_{Dapp} across the entire brain was enforced. In this present work, we chose to remove this constraint and estimate a separate K_{Dapp} for each brain region to account for variations in competing endogenous 5-HT. We utilize the rhesus monkey model to examine sex specific differences in K_{Dapp} , B_{max} , and BP_{ND} . In pursuit of this goal, the use of a group arterial function was investigated and validated for estimation of the binding parameters.

2 Methods

2.1 Radiosynthesis

The radiosynthesis of *trans*-[^{18}F]mefway (N-{2-[4-(2-methoxyphenyl)-piperazinyl]ethyl}-N-(2-pyridyl)-N-(4-*trans*-[^{18}F]-fluoromethylcyclohexane)carboxamide) was performed similar to previously reported methods (Saigal et al, 2006). Typical batch yields were between 1.5–3.0 GBq. At time of first injection, the average specific activity was 124 ± 55 GBq/ μ mol resulting in < 2% 5-HT_{1A} receptor occupancy based upon our previous work (Wooten et al, 2012). The unlabeled mefway used for the partial saturation doses was purchased from a commercial vendor (Huayi Isotopes, Toronto, ON, Canada) as an 85:15 isomeric mixture of *trans*-mefway to *cis*-mefway. Our previous work has shown the *cis*-mefway component of the mixture to offer negligible competition to *trans*-mefway 5-HT_{1A} binding and was therefore omitted from the calculation of injected mass for compartmental modeling purposes (Wooten et al, 2011b).

2.2 PET Scans

2.2.1 Subjects—MI PET experiments were completed on a total of 17 (6 male, 11 female; 10.4 ± 1.7 kg) *Macaca mulatta* (rhesus) subjects of 16.4 ± 1.2 years of age (human equivalent of approximately 50 years of age). The subjects were selected from an ongoing research program investigating the role of prenatal stressors on neurodevelopment (see Schneider et al., 1999 for details). For this work, subjects were drawn from a group receiving prenatal acoustic stress (N=10; 4 male, 6 female) and a group of age matched controls (N=7; 2 male, 5 female). Following birth, all subjects were mother reared for the first 6 months of life. The subjects were then housed with peer groups until 30 months of age and then in pairs of similar age, sex, and prenatal condition. Due to the experimental design in creating this cohort (i.e. pregnant females were selected prior to knowledge of the offspring sex), the distribution of male and female subjects were determined by birth.

For the scanning procedures, the subjects were initially anesthetized using ketamine (10 mg/kg, IM) administered no less than 40 minutes before first bolus injection. Atropine sulfate (0.27 mg, IM) was administered to reduce secretions during the scanning procedures. Anesthesia was maintained throughout the duration of the PET experiment using isoflurane (1–2%). Heart rate, body temperature, breathing rate, and oxygen saturation levels were monitored and recorded. A catheter was placed in the saphenous vein for administration of radiotracer and a second catheter was placed in the femoral artery for arterial sampling. The subjects were removed from the scanner following the data acquisition and returned to their cages when swallowing reflexes were restored and monitored until fully alert. Subjects were housed in the Harlow Primate Laboratory at the University of Wisconsin-Madison. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

2.2.2 Scanning Protocol—The three-injection scanning protocol including injection times and specific activities were based upon optimizations, performed to increase identifiability of K_{Dapp} and B_{max} , as described in our previous work (Wooten et al, 2012). Injection times, average specific activities, and average injected activities for males and females are shown in Table 1. To allow estimation of BP_{ND} , the first injection consisted of

high specific activity [^{18}F]mefway with a 90 minute scanning duration. For the second (partial saturation) injection, the average unlabeled *trans*-mefway mass per subject weight was 12 ± 4 nmol/kg and 12 ± 5 nmol/kg for male and female subjects, respectively. Our previous work demonstrated 12 nmol/kg of *trans*-mefway corresponded to a peak transient occupancy of approximately 75%. The third injection was administered at 130 minutes and a total scanning duration of 180 minutes was acquired.

A Concorde microPET P4 scanner was used for the scanning procedures (Tai et al, 2001). The microPET P4 has a detection sensitivity of $\sim 1.2\%$ and an in-plane resolution of 2.8 mm as measured by the reconstruction procedures used in this protocol. Subjects were placed in a custom built stereotaxic head holder in the prone position. Prior to injection of radiotracer, a 518 second transmission scan was performed using a ^{57}Co point source. Emission data collection was initiated with the first bolus injection of [^{18}F]mefway and was acquired continuously throughout the duration of the experiment. Each of the three bolus injections were given in a 5 mL volume over 30 seconds.

2.2.3 Arterial Plasma Sampling—Arterial plasma sampling of [^{18}F]mefway was performed to provide an input function for compartmental modeling. At the time of first injection, arterial sampling was initiated with sampling intervals of approximately 10 seconds for the first 2 minutes, then increasing to every 10 minutes by the end of an injection duration. In the case that arterial sampling was not performed, late venous samples were obtained at 10 minute intervals beginning at 20 minutes after an injection to allow adequate time for [^{18}F]mefway concentration to equilibrate between arterial and venous pools. Venous sampling was not incorporated in compartmental modeling and was not acquired in studies with arterial sampling. Whole blood samples were mixed with 50 μL of heparinized saline and assayed for radioactivity using a 2" NaI(Tl) well counter that was cross calibrated with the PET scanner. The whole blood samples were then centrifuged for 5 minutes to extract 250 μL of plasma which was subsequently added to 50 μL of sodium bicarbonate solution. The lipophilic [^{18}F]mefway was extracted from the plasma using two liquid-liquid ethyl acetate extractions. Extraction was performed similar to our previous work that validated no significant lipophilic metabolites of [^{18}F]mefway using thin layer chromatography (Saigal et al, 2006; Wooten et al, 2011a). The ethyl acetate extractions were then assayed for radioactivity which resulted in a plasma radioactivity concentration of [^{18}F]mefway in units of kBq/mL. The same method of [^{18}F]mefway radioactivity concentration assay was used for the venous samples.

2.3 Data Analysis

2.3.1 Image Reconstruction—Dynamic list mode emission data were binned into sinograms of two minute duration for the entire time series. Sinograms were reconstructed using filtered backprojection and a 0.5 cm^{-1} ramp filter with corrections applied for attenuation, scatter, and scanner normalization. The final matrix size of the output image was $128 \times 128 \times 63$, with voxel dimensions of $1.90 \times 1.90 \times 1.21\text{ mm}^3$. The images were denoised using the HYPR-LR algorithm and a $3 \times 3 \times 4$ voxel filtering kernel (Christian et al, 2009).

2.3.2 Regions of Interest—A standard space template of [^{18}F]mefway binding was created using a population-average MRI-based atlas of the rhesus macaque (McLaren et al, 2009). The reconstructed PET images were spatially normalized to the [^{18}F]mefway template volume. Only brain regions with high 5-HT_{1A} receptor density were chosen for this analysis to maximize the precision of the binding parameter estimates (discussed in section 2.3.4). Regions of interest (ROIs) were generated from a rhesus atlas developed in house which was based upon Paxinos et al (1999). ROIs were placed in the areas of the

hippocampus (Hp: 1.6 cm³), dorsal anterior cingulate cortex (dACC: 0.8 cm³), amygdala (Am: 0.2 cm³), and raphe nuclei (RN: 0.1 cm³) to allow the extraction of time activity curves (TACs). A rigid body coregistration was used to align ROIs (Hp, dACC, Am) to each individual subject and the coregistered ROIs from each subject visually inspected. To minimize variability of ROI positioning in the RN, spherical fixed volume (0.1cm³) ROIs were manually centered over the high focal binding area which included portions of the dorsal and medial regions. ROIs were also drawn in the cerebellum (CB: 1.0 cm³) including both gray and white matter avoiding the vermis (similar to Wooten et al, 2011b). Exclusion of the venous sinus in the CB ROI by a margin of several mm was carefully verified by examining ROI placement on the early and late PET frames. TACs were then extracted to serve as a reference for BP_{ND} estimation.

2.3.3 Group Input Function—A group input function (GIF) was used in compartmental modeling for estimation of kinetic parameters. Construction of the GIF is discussed in the Appendix. To test the validity of the GIF, parameter estimates found using the GIF were compared to parameter estimates obtained using the 7 subject specific input functions. Estimates found using the two different input functions were compared using total least squares regression analysis and the mean absolute percentage error (MAPE). The

MAPE = $\left(\frac{1}{N} \sum_{n=1}^N \left| \frac{\theta_{P(n)} - \theta_{GIF(n)}}{\theta_{P(n)}} \right| \times 100 \right)$ where n = 1 to N and N(=7) is the total number of subjects, θ represents the subject's parameter estimate found using the individual input function (θ_P) and GIF (θ_{GIF}).

2.3.3 Parameter Estimation—Compartmental modeling was performed to acquire region specific parameter estimates of receptor density (B_{max}), bidirectional rate constants of tracer across the blood brain barrier K_1 (mL/min/mL) and k_2 (min⁻¹), association rate constant (k_{on}) (min⁻¹), and dissociation rate constant (k_{off}) (min⁻¹). Our model did not attempt to measure differences in endogenous 5-HT, which are inherent to k_{on} through the relation:

$$k_{on} = (k_{on})^0 / \left(1 + [5HT] / K_D^{en} \right) \quad (1)$$

where k_{on} represents the association constant in the presence of endogenous 5-HT (as measured in these experiments), $(k_{on})^0$ is the association rate resulting in the absence of endogenous 5-HT, [5-HT] is the synaptic concentration of 5-HT, and K_D^{en} is the equilibrium dissociation constant of 5-HT (Delforge et al, 2001).

A two compartment model was used to account for ligand in free (F) (including nondisplaceably bound) and specifically bound (B) compartments, both in units of pmol/mL. The state equations describing the time dependent molar concentrations in each compartment are given by:

$$dt = K_1 C_{Pi}^* - k_2 F_i + k_{off} B_i - k_{on} F_i \left(B_{max} - \sum_i B_i \right) \quad (2)$$

$$dt = k_{on} F_i \left(B_{max} - \sum_i B_i \right) - k_{off} B_i \quad (3)$$

where C_{Pi}^* (pmol/mL) represents the GIF for each injection ($i = 1$ to 3). The time course of the F and B compartment concentrations can then be summed to model the data as acquired by the PET scanner, given by the equation:

$$PET_{model}(t) = \frac{1}{t_j - t_{j-1}} \int_{t_{j-1}}^{t_j} \left\{ \sum_i sa_i(t) (1 - f_v) (F_i(t) + B_i(t)) + f_v A_i \right\} dt \quad (4)$$

where t_j represents the end time of each PET frame, ($sa_i(t)$) is the time decaying specific activities, f_v is the fractional blood volume ($f_v = 0.04$) and A_i is the arterial whole blood concentration.

Compartmental modeling and parameter estimation was performed using COMKAT (Muzic and Cornelius, 2001). Parameter estimates (K_1 , k_2 , k_{on} , k_{off} , and B_{max}) were made separately for each region (Hp, dACC, Am, RN) and used to calculate the nondisplaceable distribution volume, $V_{ND} = K_1/k_2$ and $K_{Dapp} = k_{off}/k_{on}$. This is distinct from our previous work which assumed K_{Dapp} was constant across all regions. Parameter estimates were obtained by minimizing the least squares objective function ($o.f.$) between measured ROI PET data (PET_{meas}) and modeled PET data (PET_{model}) over each frame (j), using uniform weighting (w_j), as described:

$$o.f. = \sum_{j=1}^J W_j (PET_{modelj} - PET_{measj})^2 \quad (5)$$

Parameter uncertainties were estimated using Monte Carlo methods (similar to: Salinas et al, 2007 and Vandehey et al, 2010). In short, noise free PET data was simulated using parameter estimates and C_{Pi}^* . Gaussian noise realizations (50) were generated and added to the noise free PET data to simulate noise observed in measured PET data. Parameter estimates for the 50 noise realizations were then obtained to acquire coefficients of variation ($CV = 100 \times s.d./mean$).

2.3.4 Estimation of Binding Potential—The nondisplaceable binding potential (BP_{ND}) (Innis et al, 2007) was calculated using radioactive decay corrected data from the first injection duration (high specific activity 90 minute duration). The BP_{ND} was estimated using the multilinear reference tissue model (MRTM) (Ichise et al, 1996) to calculate the distribution volume ratio (DVR). The CB was used as a reference region and BP_{ND} was calculated as: $BP_{ND} = DVR - 1$.

2.3.5 Statistical Analysis—The Wilcoxon's rank-sum test was used to test the null hypothesis of no difference between the distribution of the males and females populations. Sex differences were considered significant for *p-values < 0.05 and were checked for multiple comparisons using Holm's method (Holm, 1979). Differences between the treatment (prenatal stress) and control groups were tested using Wilcoxon's rank-sum prior to performing the analysis between males and females. No statistically significant differences were found between the two treatment groups (prenatal stress and controls) for the regions examined and these groups were therefore combined in the statistical model.

3 Results

3.1 Group Input Function

The comparison of in vivo binding parameter estimates using C_p^* versus C_p revealed MAPEs of less than 10% in the MTC, dACC, and Am (B_{max} : 7%, 5%, 8%; K_{Dapp} : 7%, 9%,

9%) and slightly higher MAPE in the RN ($B_{\max}$: 15%; K_{Dapp} : 12%). Total least squares regression performed between C_p or C_p^* derived estimates of $B_{\max}$ and K_{Dapp} over all the regions are displayed in Figure 1, resulting in slopes of 0.95 and 0.97 for $B_{\max}$ and K_{Dapp} , respectively.

3.2 Male and Female Binding Parameter Estimates

The results of the sex-based differences for K_{Dapp} , $B_{\max}$, and BP_{ND} are shown in Table 2. Median K_{Dapp} values were lower for female subjects in all regions. Significant sex differences in K_{Dapp} were found in: Hp (−32%, $p=0.014$), Am (−38%, $p=0.020$), and RN (−37%, $p=0.007$) and a trend was seen in the dACC (−27%, $p=0.06$). In all regions, $B_{\max}$ was higher in males than females. However, the sex differences in $B_{\max}$ measurements were only significant in the Hp with a difference of −29% ($p=0.027$). The BP_{ND} measure showed significant differences in the Am (14%, $p=0.047$) and RN (23%, $p=0.014$), however, this was not found in the Hp or dACC. Across the regions, the average intersubject CV(=s.d./mean) was 31%, 26%, and 21% for K_{Dapp} , $B_{\max}$, and BP_{ND} , respectively, suggesting K_{Dapp} and $B_{\max}$ show only slightly higher variation than BP_{ND} . Uncertainties in parameter estimates found using Monte Carlo methods, given as CV, were 5.7% and 6.6% for K_{Dapp} and $B_{\max}$, respectively, averaged across all regions.

4 Discussion

The 5-HT_{1A} receptor plays an important role in regulating the 5-HT system. Sex-dependent variations in the 5-HT_{1A} system may be a source of the functional differences in the 5-HT system between males and females. In this work, we report on the sex-specific differences of in vivo 5-HT_{1A} receptor density ($B_{\max}$), ligand-receptor dissociation constant (K_{Dapp}), and [¹⁸F]mefway BP_{ND} . Knowledge of these characteristics and the methodologies developed for their measurement may ultimately aid in examining the underlying causes of 5-HT_{1A} dysfunction and their contribution to neuropsychiatric illnesses.

The high level of consistency of [¹⁸F]mefway plasma time course observed in the initial subjects led us to explore the use of a group input function which is discussed further in the Appendix. Analysis showed that use of the GIF in place of an individual subject's input function yielded reliable results as indicated by the low MAPE and the high consistency of parameter estimates shown in Figure 1. The relatively small y-intercept and slopes close to unity in Figure 1 indicate high levels of uniformity between the two methods. Although the y-intercept of $B_{\max}$ was within the uncertainty of the fit, the y-intercept of K_{Dapp} wasn't, indicating a small bias caused by the GIF.

4.1 $B_{\max}$ and K_{Dapp} Estimation

A significant saturation of receptors must be achieved to effectively uncouple the binding parameters k_{on} and $B_{\max}$ (Salinas et al, 2007; Wooten et al, 2012). Our previous work indicated receptor occupancy of ~75% was optimal for uncoupling the binding parameters, corresponding to a dose of approximately 12 nmol/kg of *trans*-mefway. In this study, roughly equal doses of *trans*-mefway were given to the male and female subjects in injection 2, 12 ± 4 and 12 ± 5 nmol/kg, respectively.

The estimated uncertainties derived from Monte Carlo methods in our previous work were 6% for K_{Dapp} and 3–4% for $B_{\max}$ (Wooten et al, 2012). The estimated uncertainties in K_{Dapp} were similar in this work, however, uncertainties in $B_{\max}$ were slightly higher at 6.6% due to the estimation strategies implemented. In our previous work, the parameters were estimated using a constant K_{Dapp} across regions, thus constraining the ratio k_{on} and k_{off} in all brain areas (Wooten et al, 2012). This constraint was enforced to reduce the number of estimated

parameters and increase identifiability in the lower receptor density regions. Because the focus of this current work was to compare both 5-HT_{1A} receptor density and the effects of endogenous 5-HT competition between sexes, we chose to remove this constraint of a constant K_{Dapp} throughout brain regions. As a result, the low 5-HT_{1A} receptor density regions were not analyzed because identifiability of B_{max} and K_{Dapp} was limited in these regions. The K_1 , k_2 , k_{on} , k_{off} , and B_{max} parameters could be accurately identified in the high binding regions to provide regionally independent measures.

In our previous work we found a lack of correlation between [¹⁸F]mefway BP_{ND} and 5-HT_{1A} B_{max} indicating BP_{ND} is not necessarily an accurate proxy of B_{max} alone (Wooten et al, 2012). Similarly in this work, no correlation was found (in any ROI) between B_{max} and BP_{ND} across all of the subjects. Correlations, however, were found between reference region based BP_{ND} and the compartmental model based BP: ($BP_{model} = B_{max}/K_{Dapp}$) across all subjects in all ROIs. Further, no correlation was found between K_{Dapp} and B_{max} when examined within the male and female groups separately, as is often seen when binding parameters are not fully uncoupled (Morris et al, 1999). In agreement with our earlier findings, these results suggest [¹⁸F]mefway BP_{ND} is a representative metric for B_{max}/K_D .

MI studies alter the number of receptors available for binding, permitting an uncoupling of k_{on} and B_{max} . Figure 2 illustrates the sensitivity of the MI technique to variations in the radiotracer kinetic behavior. Simulations were performed to examine the effects of changes in K_{Dapp} (via k_{on} and k_{off}) and B_{max} on the PET response curves. Our results indicated sex-specific differences in K_{Dapp} of 30%, which could be the consequence of a $\pm 30\%$ difference in either k_{on} , k_{off} or a combination of the two. As seen in the figure, a 30% reduction in k_{on} is indistinguishable from a 30% reduction in B_{max} during the first injection period (time = 0–90 minutes). It is not until the second injection of partial saturating dose (time > 90 minutes) that k_{on} and B_{max} become distinguishable. The first 90 minutes of data illustrate the complete coupling of k_{on} and B_{max} , similar to all high-specific activity single injection PET studies. A partial saturating dose of mefway in the second injection changes the number of receptors available for binding as seen in the curves for time greater than 90 minutes. After injection 3 (high specific activity), the number of receptors available for binding are beginning to recover as illustrated by the decreased slope in the PET curves. The increase in k_{off} is observed immediately in the first injection period as it is distinguishable from PET_{model} because it represents a faster dissociation rate after the first bolus injection.

4.2 5-HT_{1A} Sex Differences

These results with [¹⁸F]mefway are similar to previously reported studies finding higher BP_{ND} values in female subjects (Parsey et al, 2002; Jovanovic et al, 2008). However, the higher BP_{ND} was not due to larger B_{max} values in female subjects, as could be suggested by the relation: $BP_{ND} \propto B_{max}/K_{Dapp}$. Instead, our results indicate K_{Dapp} is the primary source of the higher BP_{ND} for female subjects. Our results are similar to a previous study using a two-injection scatchard method which revealed higher B_{max} and K_D estimates in male subjects, however, due to high variance across the subjects the differences did not reach significance (Spinelli et al, 2010). Knowing that K_{Dapp} is a function of both k_{on} and k_{off} , further examination of these parameters revealed female subjects to have significantly higher k_{on} than male subjects in all areas (Hp: $p=0.005$; dACC: $p=0.047$; Am: $p=0.002$; RN: $p=0.005$) and no significant differences in k_{off} in any brain regions. Higher k_{on} estimates in female subjects could be due to higher receptor-ligand affinity or lower levels of endogenous serotonin competition at the receptor site (equation 1) when compared to male subjects. However, previous studies using [¹¹C]WAY-100635 to examine changes in 5-HT levels have been inconclusive (Paterson et al, 2010). Because the in vivo kinetics of [¹⁸F]mefway and [¹¹C]WAY-100635 are similar (Wooten et al, 2011a), we cannot conclude that the differences are exclusively due to endogenous 5-HT competition. Reported

extracellular basal levels of 5-HT as measured with microdialysis are 0.04–3.9 nM (Paterson et al, 2010). Interpolating the inhibition curve of [^{18}F]mefway (Saigal et al, 2006), a 4 nM concentration of serotonin would cause a roughly 10% reduction in [^{18}F]mefway binding. Additionally, lower concentrations of 5-HT at the 5-HT_{1A} autoreceptor sites in the RN should result in higher 5-HT synthesis. Studies designed to measure effects in changes in endogenous 5-HT on binding of [^{18}F]mefway are needed to fully characterize the sensitivity of [^{18}F]mefway to these changes and to identify the mechanism behind these sex-based differences in K_{Dapp} . Although male and female differences in arterial [^{18}F]mefway clearance were not observed in our subjects, we must also acknowledge the possibility of effects from sex based differences in the plasma free fraction (f_p) or tissue free fraction (f_{ND}) on our results. Further studies will be needed to examine differences in f_p between male and female subjects.

We recently reported on the effects of the serotonin-transporter-linked polymorphic region (5-HTTLPR) on the binding of [^{18}F]mefway in 58 rhesus monkeys (Christian et al, In Press). Using a large cohort with 36 females and 22 males, a 5-HTTLPR group difference on [^{18}F]mefway binding was found in the raphe nuclei and some cortical regions, with (*l/l*) homozygotes having approximately a 7% higher BP_{ND} compared to short (*s*) allele carriers. In this work we performed Wilcoxon's rank-sum test to detect if any statistically significant differences exist between the distributions of *l/l* and *s* variants. Since the statistical tests performed did not detect any significant differences and because our sample size was fairly small, we have not adjusted for genotype in our analysis. Thus, combining *l/l* and *s*-carriers seems reasonable for this specific cohort.

5 Conclusions

This work presents measurements of K_{Dapp} , B_{max} , and BP_{ND} in both male and female rhesus subjects to allow parameter comparisons between the sexes. Our results indicate that differences in BP_{ND} are primarily due to differences in K_{Dapp} , which could be due to radioligand receptor affinity or an effect of sex-specific differences in endogenous serotonin levels. Further endogenous serotonin depletion or competition studies are needed to determine its effect on the measurement of K_{Dapp} , B_{max} , and BP_{ND} . However, this study suggests measurements of B_{max} and K_{Dapp} separately are more sensitive to detecting changes and group differences in 5-HT_{1A} function.

Acknowledgments

The authors would like to thank the following for their contribution to this research: Dr. Jonathan Engle and Professor R. Jerry Nickles for assistance with isotope production; Andrew Higgins for assistance with image analysis; Julie Larson and the staff at the Harlow Center for Biological Psychology at the University of Wisconsin for nonhuman primate handling; Dr. Alex Converse and Professor Jim Holden for technical discussions; NIH grants: MH086014, AA12277, AG030524, T32CA009206. Additional support was provided by NIH grants S10RR015801, P30HD003352

This work was supported by NIH grants: MH086014, AA1 2277, AG030524, T32CA009206, P30HD003352.

Appendix

Appendix: Group Input Function

The initial analysis of the plasma [^{18}F]mefway time courses from the initial studies showed a high level of consistency between the subjects. Therefore, a GIF was constructed using the arterial plasma [^{18}F]mefway data from these subjects to determine if arterial sampling was necessary for the remaining studies. This appendix describes the construction and validation of a group input function for estimation of 5-HT_{1A} B_{max} and [^{18}F]mefway K_{Dapp} .

Construction of the Group Input Function

The MI compartmental modeling requires each input function to be uniquely defined for every injection. The residual activity from prior injections must be mathematically removed from subsequent injections. Starting with arterial plasma sampling data of [^{18}F]mefway in units of kBq/mL, a bi-exponential function ($a_1e^{-\lambda_1t} + a_2e^{-\lambda_2t}$) was fit to times > 5 minutes after a bolus injection to permit extrapolation of [^{18}F]mefway clearance from the plasma which allows its subtraction from later injections. Full arterial sampling over the entire 3-injection 180 minute PET scan was performed in 7 out of the 17 total subjects in this study. A group input function (GIF) was constructed using 11 full arterial data sets (3 male, 8 female; 7 stress, 4 controls) which included 7 from this study and 4 additional data sets not included in the sex-based comparisons due to unsatisfactory experimental design. The 4 additional data sets were from subjects in the same cohort and were age-matched. First, the data sets were normalized by subject mass and injected activity. The normalized arterial time courses for each of three injections from each subject were then averaged together and fit to a 3-exponential function (Feng et al, 1993) to form separate arterial functions. These functions (one for each of three injections) were then corrected for each subject using their own mass, injected activities, and specific activities (at the time of each injection) resulting in the subject specific GIF in units of pmol/mL of mefway.

Validation of the Group Input Function

Validation of the group input function included sex-specific comparison of male and female [^{18}F]mefway plasma time courses and comparisons with late venous samples. Sex-based differences were carefully examined in the arterial blood time course and metabolism profile. An analytic function was fit to averaged male (N=3) and female (N=8) [^{18}F]mefway plasma time course curves to allow a comparison of initial fast washout and late washout rates. In studies without arterial data (N=10), venous data was compared to arterial data for the identification of outliers or subjects with drastically different rates of mefway clearance or blood scaling. Venous data, however, was not incorporated into the modeling procedure.

The use of a GIF has the potential to introduce bias in the parameter estimates if it is not an accurate representation of an individual's input function. To explore the extent of bias caused by misrepresentative input functions, an examination of the sensitivity of two GIF characteristics on the outcome parameter estimates was conducted; 1) overall scaling changes in the arterial time course and 2) clearance rate differences of [^{18}F]mefway from the plasma. As implemented, the GIF was calculated by scaling the individual arterial samples by subject mass and injected activity. In these studies, body fat percentage was not taken into account and has the potential to introduce blood scaling errors due to a lack of linearity between blood volume and subject mass. Similarly, the clearance rate of tracer from the individual subjects caused by differences in tracer metabolism could lead to error in the GIF. Simulated error in the group input functions is shown in Figure 4A corresponding to either scaling (left) or clearance rate (right) error. The left panel of Figure 4 (top) shows the scaling

error that applied to the GIF ($\alpha \times C_{P(i=1-3)}^*$) where the scaling error (α) ranged between 0.5 – 2.0 representing 50% to 200% of the true value. Figure 4A (top right panel) also displays the first order clearance error ($e^{-\beta t} \times C_{P(i=1-3)}^*$) that was applied to the GIF which is similar to methods reported in the literature (Normandin and Morris, 2008). The effects of the input function error on the parameter estimates for V_{ND} , K_{Dapp} , and B_{max} were then examined.

Group Input Function Validation Analysis

Male and female separated plasma time courses are shown in Figure 3A. No statistically significant differences in [^{18}F]mefway arterial plasma clearance were observed between the

sexes. The separated male and female averaged arterial functions had similar initial, fast components of [^{18}F]mefway clearance ($\lambda_1^{\text{male}}=1.7 \text{ min}^{-1}$; $\lambda_1^{\text{female}}=1.9 \text{ min}^{-1}$). A non-significant difference of <3% in the slow clearance term (λ_3) was observed, indicating similar slow washout between male and female subjects. By simulating comparable changes in the arterial input function, it was found that a 3% percent change in λ_3 results in approximately a 1% change in B_{max} and a 2% change in K_{Dapp} . A comparison of the late venous plasma [^{18}F]mefway time course with the arterial sampling data is shown in Figure 3B. The late ($t>50$ minutes) venous measured concentrations are generally within the variation seen in arterial time courses, indicating no significant outliers.

Effects of changes in scaling and clearance rates in the GIF are shown in Figure 4B. Errors from scaling the GIF have a large effect on V_{ND} , but negligible influence on K_{Dapp} and B_{max} , whereas inaccuracies in the clearance rate of the GIF have a more profound effect on K_{Dapp} and B_{max} estimates. For the 7 subjects reported herein, the average parent [^{18}F]mefway fraction in plasma at 90 minutes was 15%, and ranged from 10% to 22% (represented by the dashed lines in Figure 4B). This range of clearance error would lead to a -7% to +3% error in K_{Dapp} and -3% to +6% error in B_{max} . The scaling and clearance terms do not address all of the potential uncertainty in the GIF, however, these analyses illustrate that the variations present in our measured input functions do not propagate into large uncertainties in our outcome measures of K_{Dapp} and B_{max} .

Finally, using the GIF in place of the individual input function gave an average MAPE for both K_{Dapp} and B_{max} of <10%, which is much less than the overall reported sex-based differences of greater than 30% for K_{Dapp} . Based upon this one study, we cannot advocate the general use of a GIF for other species or cohorts and acknowledge that the high consistency among these results is likely due to the close age range and similar lifestyle conditions of the subjects.

References

- Arango V, Underwood MD, Gubbi AV, Mann JJ. Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims. *Brain Res.* 1995; 688:121–133. [PubMed: 8542298]
- Biver F, Lotstra F, Monclus M, Wikler D, Damhaut P, Mendlewicz J, Goldman S. Sex difference in 5-HT₂ receptor in the living human brain. 1996; 204:25–28.
- Cahill L. Why sex matters for neuroscience. *Nat. Rev. Neurosci.* 2006; 7:477–484. [PubMed: 16688123]
- Christian BT, Vandehey NT, Floberg JM, Mistretta CA. Dynamic PET denoising with HYPR processing. *J Nucl. Med.* 2010; 51:1147–1154. [PubMed: 20554743]
- Christian BT, Wooten DW, Hillmer AT, Tudorascu DL, Converse AK, Moore CF, Ahlers EO, Barnhart TE, Kalin NH, Barr CS, Schneider ML. Serotonin transporter genotype affects serotonin 5-HT_{1A} binding in primates. *J. Neurosci.* In Press.
- Cosgrove KP, Mazure CM, Staley JK. Evolving knowledge of sex differences in brain structure, function and chemistry. *Biol. Psychiatry.* 2007; 62:847–855. [PubMed: 17544382]
- Delforge J, Bottlaender M, Pappata S, Loc'h C, Syrota A. Absolute quantification by positron emission tomography of the endogenous ligand. *J. Cereb. Blood Flow Metab.* 2001; 21:613–630. [PubMed: 11333372]
- Dillon KA, Gross-Isseroff R, Israeli M, Biegon A. Autoradiographic analysis of serotonin 5-HT_{1A} receptor binding in the human brain postmortem: effects of age and alcohol. *Brain Res.* 1991; 554:56–64. [PubMed: 1834306]
- Feng , Huang SC, Wang X. Models for computer simulation studies of input functions for tracer kinetic modeling with positron emission tomography. *Int. J. Biomed. Comput.* 1993; 32:95–110. [PubMed: 8449593]

- Ichise M, Ballinger JR, Golan H, Vines D, Luong A, Tsai S. Noninvasive quantification of dopamine D2 receptors with iodine-123-IBF SPECT. *J. Nucl. Med.* 1996; 37:513–520. [PubMed: 8772658]
- Holm S. A simple sequentially rejective multiple test procedure. *Scand J Statist.* 1979; 6:65–70.
- Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang S-C, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J. Cereb. Blood. Flow. Metab.* 2007; 27:1533–1539. [PubMed: 17519979]
- Jovanovic H, Lundberg J, Karlsson P, Cerin A, Saijo T, Varrone A, Halldin C, Nordström AL. Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. *NeuroImage.* 2008; 39:1408–1419. [PubMed: 18036835]
- Maron E, Töru I, Hirvonen J, Tuominen L, Lumme V, Vasar V, Shlik J, Nutt DJ, Helin S, Nägren K, Tiitonen J, Hietala J. Gender differences in brain serotonin transporter availability in panic disorder. *J. Psychopharmacol.* 2010; 25:952–959. [PubMed: 21148024]
- Matsubara S, Arora RC, Meltzer HY. Serotonergic measures in suicide brain: 5-HT_{1A} binding sites in frontal cortex of suicide victims. *J. Neural Transm. Gen.* 1991; 85:181–194.
- McLaren DG, Kosmatka KJ, Oakes TR, Kroenke CD, Kohama SG, Matochik JA, Ingram DK, Johnson SC. A population-average MRI-based atlas collection of the rhesus macaque. *Neuroimage.* 2009; 45:52–59. [PubMed: 19059346]
- Meltzer CC, Drevets WC, Price JC, Mathis CA, Lopresti B, Greer PJ, Villemagne VL, Holt D, Mason NS, Houck PR, Reynolds CF III, DeKosky ST. Gender-specific aging effects on the serotonin 1A receptor. *Brain Res.* 2001; 895:9–17. [PubMed: 11259754]
- Morris ED, Bonab AA, Alpert NM, Fischman AJ, Madras BK, Christian BT. Concentration of dopamine transporters: to B_{max} or not to B_{max}? *Synapse.* 1999; 32:136–140. [PubMed: 10231133]
- Muzic RF, Cornelius S. COMKAT: Compartment model kinetic analysis tool. *J. Nucl. Med.* 2001; 42:636–645. [PubMed: 11337554]
- Nishizawa S, Benkelfat C, Young SN, Leyton M, Mzengeza S, de Montigny C, Blier P, Diksic M. Differences between males and females in rates of serotonin synthesis in human brain. *Proc. Natl. Acad. Sci.* 1997; 94:5308–5313. [PubMed: 9144233]
- Normandin MD, Morris ED. Estimating neurotransmitter kinetics with ntPET: a simulation study of temporal precision and effects of biased data. *Neuroimage.* 2008; 39:1162–1179. [PubMed: 18023364]
- Ortiz J, Artigas F, Gelpí E. Serotonergic status in human blood. *Life Sci.* 1988; 43:983–990. [PubMed: 2459577]
- Palego L, Marazziti D, Rossi A, Giannaccini G, Naccarato AG, Lucacchini A, Cassano GB. Apparent absence of aging and gender effects on serotonin 1A receptors in human neocortex and hippocampus. *Brain Res.* 1997; 758:26–32. [PubMed: 9203529]
- Parsey RV, Oquendo MA, Simpson NR, Ogden RT, Van Heertum R, Arango V, Mann JJ. Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT_{1A} receptor binding potential measured by PET using [¹¹C]WAY-100635. *Brain Res.* 2002; 954:173–182. [PubMed: 12414100]
- Paterson LM, Tyacke RJ, Nutt DJ, Knudsen GM. Measuring endogenous 5-HT release by emission tomography: promises and pitfalls. *J. Cereb. Blood Flow Metab.* 2010; 30:1682–1706. [PubMed: 20664611]
- Paxinos, G.; Huang, XF.; Toga, AW. *The rhesus monkey brain in stereotaxic coordinates.* 1st ed. Academic Press; San Diego: 1999.
- Saigal N, Pichika R, Easwaramoorthy B, Collins D, Christian BT, Shi B, Narayanan TK, Potkin SG, Mukherjee J. Synthesis and biologic evaluation of a novel serotonin 5-HT_{1A} receptor radioligand, [¹⁸F]-labeled mefway, in rodents and imaging by PET in a nonhuman primate. *J. Nucl. Med.* 2006; 47:1697–1706. [PubMed: 17015907]
- Sakai Y, Nishikawa M, Leyton M, Benkelfat C, Young SN, Diksic M. Cortical trapping of α- [¹¹C]methyl-l-tryptophan, an index of serotonin synthesis, is lower in females than males. *NeuroImage.* 2006; 33:815–824. [PubMed: 16996279]

- Salinas C, Muzic RF, Ernsberger P, Saidel GM. Robust experiment design for estimating myocardial beta adrenergic receptor concentration using PET. *Med. Phys.* 2007; 34:151–65. [PubMed: 17278500]
- Schneider ML, Roughton EC, Koehler AJ, Lubach GR. Growth and development following prenatal stress exposure in primates: an examination of ontogenetic vulnerability. *Child Dev.* 1999; 70:263–274. [PubMed: 10218255]
- Spinelli S, Chefer S, Carson RE, Jagoda E, Lang L, Heilig M, Barr CS, Suomi SJ, Higley JD, Stein EA. Effects of early-life stress on serotonin(1A) receptors in juvenile Rhesus monkeys measured by positron emission tomography. *Biol. Psychiatry.* 2010; 67:1146–1153. [PubMed: 20172506]
- Tai C, Chatziioannou A, Siegel S, Young J, Newport D, Goble RN, Nutt RE, Cherry SR. Performance evaluation of the microPET P4: a PET system dedicated to animal imaging. *Phys. Med. Biol.* 2001; 46:1845–1862. [PubMed: 11474929]
- Vandehey NT, Moirano JM, Converse AK, Holden JE, Mukherjee J, Murali D, Nickles RJ, Davidson RJ, Schneider ML, Christian BT. High-affinity dopamine D₂/D₃ PET radioligands [¹⁸F]-fallypride and [¹¹C]-FLB457: A comparison of kinetics in extrastriatal regions using a multiple-injection protocol. *J. Cereb. Blood. Flow. Metab.* 2010; 30:994–1007. [PubMed: 20040928]
- Wooten DW, Moraino JM, Hillmer AT, Engle JW, DeJesus OT, Murali D, Barnhart TE, Nickles RJ, Davidson R, Schneider ML, Mukherjee J, Christian BT. In vivo kinetics of [¹⁸F]MeFWAY: A comparison with [¹¹C]WAY-100635 and [¹⁸F]MPPF in the nonhuman primate. *Synapse.* 2011a; 65:592–600. [PubMed: 21484878]
- Wooten DW, Hillmer AT, Murali D, Barnhart TE, Schneider ML, Mukherjee J, Christian BT. An in vivo comparison of *cis*- and *trans*-[¹⁸F]mefway in the nonhuman primate. *Nucl. Med. Biol.* 2011b; 38:925–932. [PubMed: 21741252]
- Wooten DW, Hillmer AT, Moirano JM, Ahlers EO, Slesarev M, Barnhart TE, Mukherjee J, Schneider ML, Christian BT. Measurement of 5-HT_{1A} receptor density and in-vivo binding parameters of [¹⁸F]mefway in the nonhuman primate. *J. Cereb. Blood Flow Metab.* 2012; 32:1546–1558. [PubMed: 22472611]

Highlights

- Females displayed lower in vivo K_D than males
- Lower K_D could result from lower endogenous 5-HT competition
- BP_{ND} is not as sensitive as the uncoupled parameters B_{max} and K_D
- An [^{18}F]mefway group arterial input function was validated for this cohort

Figure 1. Group input function (C_P^*) estimates for $B_{\max}$ and K_{Dapp} vs individual input (C_P) estimates for $B_{\max}$ and K_{Dapp} over all regions.

Figure 2.

Simulated time activity curves (TACs) in the hippocampus. The TACs illustrate situations of no change in parameters (solid line), 30% decrease in k_{on} (dash dotted line) and B_{max} (dashed line), and 30% increase in k_{off} (dotted line).

Figure 3.

(A) Averaged arterial plasma [¹⁸F]mefway time courses for injection 1 over male (dotted line), female (dashed line), and all (solid line) subjects. (B) Averaged arterial plasma (dashed line) and individual late venous plasma (solid lines) [¹⁸F]mefway time courses for injection 1. Arterial data was fit to a three exponential and venous data was fit to a one exponential function for times 50–90 minutes.

Figure 4.

(A) Original GIF (solid lines) for injections 1–3 and altered GIFs (dashed and dotted lines) that result from systematic error in scaling of magnitude by α (left) or applying a clearance rate error $e^{-\beta t}$ (right). The dashed and dotted time courses represent the variation in GIF by the application of α or β as shown in B. (B) Effects on V_{ND} (top row), K_{Dapp} (middle row), and B_{max} (bottom row) caused by the simulated error in the GIF. α was varied between 0.5 – 2.0 and the clearance rate ($e^{-\beta t}$) between 0.5 – 2.0 at $t = 90$ minutes. The vertical solid lines at 1 represents the original GIF parameter estimates and the dashed lines in the right column represents the range of variation in parent $[^{18}F]$ mefway radioactivity as observed in the subjects in this study.

Table 1

Injection parameters

Injection #	Time (minutes)	Male (N=6)			Female (N=11)		
		Activity (MBq)	Mefway mass (nmol/kg)	Specific activity (GBq/ μ mol)	Activity (MBq)	Mefway mass (nmol/kg)	Specific activity (GBq/ μ mol)
1	0	58 $\pm$ 5	0.05 $\pm$ 0.01	122 $\pm$ 48	60 $\pm$ 4	0.07 $\pm$ 0.03	127 $\pm$ 66
2	90	61 $\pm$ 5	12 $\pm$ 4	0.63 $\pm$ 0.60	58 $\pm$ 4	12 $\pm$ 5	0.60 $\pm$ 0.24
3	130	60 $\pm$ 3	0.11 $\pm$ 0.04	54 $\pm$ 21	60 $\pm$ 3	0.15 $\pm$ 0.07	56 $\pm$ 29

Table 2 Regional results for K_{Dapp} , B_{max} , and BP_{ND}

		male (N=6; 1/1=3; s-carriers=3)	female (N=11; 1/1=7; s-carriers=4)		
Parameter	ROI	median (IQR)	median (IQR)	difference	p-value
K_{Dapp} (pmol/mL)	Hp	4.8 (1.3)	3.5 (1.5)	-32%	**0.014
	dACC	4.2 (1.3)	3.2 (1.4)	-27%	0.061
	Am	4.1 (0.6)	2.8 (0.9)	-38%	**0.020
	RN	5.9 (1.4)	4.1 (1.3)	-37%	**0.007
B_{max} (pmol/mL)	Hp	38.3 (7.2)	28.4 (7.5)	-29%	*0.027
	dACC	34.4 (16.9)	30.3 (6.0)	-13%	0.574
	Am	25.8 (7.8)	21.0 (4.9)	-20%	0.182
	RN	33.2 (10.9)	23.9 (8.2)	-33%	0.098
BP_{ND}	Hp	3.9 (0.9)	4.8 (1.1)	19%	0.122
	dACC	3.9 (1.0)	4.8 (1.2)	22%	0.149
	Am	4.0 (0.6)	4.7 (0.9)	14%	*0.047
	RN	2.7 (0.5)	3.4 (0.8)	23%	*0.014

Data are median and interquartile range (IQR) for each ROI by parameter and sex. Percent differences between male and female groups along with Wilcoxon's rank-sum adjusted p-values are shown.

hippocampus (Hp), dorsal anterior cingulate cortex (dACC), amygdala (Am), raphe nuclei (RN)

* p-values < 0.05 are considered statistically significant.

** p-values survived adjustment using Holm's procedure.