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First-in-Human Evaluation of ^{18}F-Mefway, a PET Radioligand Specific to Serotonin-1A Receptors

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Abstract

The serotonin-1A (5-HT$_{1A}$) receptor is implicated in an array of neurological and psychiatric disorders. Current PET radioligands targeting 5-HT$_{1A}$ receptors have limitations hindering widespread PET studies of this receptor system. The 5-HT$_{1A}$ specific antagonist radioligand *N*-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(*trans*-4-^{18}F-fluoromethylcyclohexane)carboxamide (^{18}F-mefway) exhibited promising in vivo properties in rhesus monkeys. The goal of this work was to examine the in vivo cerebral binding profile and metabolism of ^{18}F-mefway in humans.

Methods—Dynamic ^{18}F-mefway PET data were acquired for six healthy volunteers (4F, 2M; 22–38 years). Scans were initiated with the injection of 192–204 MBq radiotracer and data were acquired for two hours. Venous blood samples were collected and assayed to examine the in vivo metabolism profile of ^{18}F-mefway. To examine the test-retest variability of ^{18}F-mefway, a second PET scan was acquired at least two weeks later for four subjects. Regional binding potentials (BP$_{ND}$) were calculated with MRTM, and voxel-wise BP$_{ND}$ maps were calculated with Logan graphical analysis. Regions surrounding the brain were carefully inspected for uptake of radiolabeled species in bone.

Results—^{18}F-Mefway uptake in the brain occurred quickly with peak SUVs of 1.7. Rapid washout in the cerebellum resulted in SUVs of 0.2 at 120 minutes, while regions with specific 5-

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HT_{1A} binding exhibited retention of radioligand yielding SUVs of 0.4–0.9 at 120 minutes. Rapid metabolism of ^{18}F-mefway was observed, with no detected ^{18}F-fluoride ions in plasma. BP_{ND} values of 2.4 were measured in the mesial temporal lobe, with values of 1.6 in insular cortex and 0.7–1.0 in other cortical regions. Stable BP_{ND} estimates were obtained using 90 minutes of dynamic data. Average test-retest variability was 8%. No evidence of radioactivity uptake in bone was observed.

Conclusion—^{18}F-Mefway exhibits favorable in vivo properties for serotonin 5-HT_{1A} receptor measurements in humans. The simple radiosynthesis, high specific binding profile, and absence of PET signal in bone make ^{18}F-mefway an attractive radiotracer for PET experiments examining the 5-HT_{1A} receptor in neuropsychiatric disorders and drug intervention.

Keywords

PET; ^{18}F-mefway; serotonin-1A; hippocampus

Introduction

The neurotransmitter 5-hydroxytryptamine (5-HT; serotonin), is a crucial regulator of many cognitive processes including memory, learning, and mood. The 5-HT_{1A} receptor is a G-protein coupled receptor that plays a vital role in regulating 5-HT transmission. These receptors occur presynaptically as autoreceptors in the raphe nuclei (1) and postsynaptically in cortical and hippocampal regions (2). Brain regions rich in 5-HT_{1A} receptor concentrations include the mesial temporal lobe, cingulate cortex, raphe nuclei, frontal cortex, and parietal cortex. The 5-HT_{1A} receptor is implicated in a variety of neuropsychiatric pathologies, including schizophrenia, Alzheimer's disease, depressive disorders, and alcohol dependence.

An important experimental technique for in vivo interrogation of 5-HT_{1A} receptors is positron emission tomography (PET) imaging. To date, the most commonly used PET antagonist radioligand for 5-HT_{1A} receptors is ^{11}C-WAY-100635 (3). This radioligand exhibits high signal in regions of specific binding relative to the cerebellum and suitable BP_{ND} quantification, however, widespread use of this radioligand has been limited. The radiochemical production for ^{11}C-WAY-100635 is difficult to reliably perform, while the short 20 minute half-life of the ^{11}C label requires an on-site cyclotron and yields poor counting statistics towards the end of scanning procedures. To overcome these issues, a variety of WAY-100635 analogs with the longer lived ^{18}F label (110 minute half-life) have been developed (for review see 4). ^{18}F-MPPF has been successfully used to study 5-HT_{1A} physiology in human subjects, but suffers from poor brain penetration and subsequently yields low target-to-background ratios (5). ^{18}F-FCWAY has kinetic properties similar to ^{11}C-WAY-100635 and a simple labeling procedure (6). However, defluorination of ^{18}F-FCWAY in vivo resulted in bone uptake of ^{18}F-fluoride ions, complicating analysis of PET data (7) and requiring enzyme inhibitors to enable suitable quantification (8).

The radioligand *N*-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-^{18}F-fluoromethylcyclohexane)carboxamide was designed to provide an ^{18}F labeled analog of ^{11}C-WAY-100635 with improved stability by moving the radiolabel from an aromatic

ring to a primary carbon. Studies of the *cis*- and *trans*- isomers of this radioligand revealed high specificity of the *trans* isomer for 5-HT_{1A} receptors (9), therefore this human study focused on the *trans* isomer, shown in Figure 1 (henceforth abbreviated as ^{18}F-mefway). ^{18}F-Mefway is produced with high yields (10), and preclinical experiments demonstrated comparable kinetic properties between ^{11}C-WAY-100635 and ^{18}F-mefway in rhesus monkeys with no evidence of defluorination (11).

Studies investigating 5-HT_{1A} receptor physiology in nonhuman primates using ^{18}F-mefway have also been performed in our labs. These findings include sex-based differences in 5-HT_{1A} function, where increased in vivo affinity of ^{18}F-mefway for the 5-HT_{1A} receptor and decreased 5-HT_{1A} binding potentials in females relative to males were observed (12). Additionally, decreased 5-HT_{1A} binding levels in 5-HTTLPR *s*-carriers (13), and increased 5-HT_{1A} binding levels following chronic alcohol self-administration have been reported (14). These ^{18}F-mefway studies therefore indicated great promise of a suitable ^{18}F-labeled radioligand to image 5-HT_{1A} specific physiology in humans.

The goal of this work was to evaluate the in vivo properties of ^{18}F-mefway in humans for the first time. The regional distribution of ^{18}F-mefway uptake and binding in the human brain is reported, including a detailed inspection of ^{18}F-mefway binding in the important high 5-HT_{1A} density region of the mesial temporal lobe. Furthermore, a preliminary analysis of ^{18}F-mefway's behavior in venous plasma samples is performed.

Materials and Methods

Subjects

Subjects were healthy volunteers consisting of four females and two males, ranging in age from 22–38 years, recruited at the University of Wisconsin-Madison. The University of Wisconsin Institutional Review Board approved all study procedures. All subjects provided informed signed consent before participation. Anti-depressive medication was verbally screened for as an exclusion criterion.

Scanning Procedures

^{18}F-Mefway was produced following previously published methods (9). The synthesis consisted of a nucleophilic substitution of the precursor, *N*-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(*trans*-4-tosyloxymethylcyclohexane)carboxamide (*tosyl-trans*-mefway; Huayi Isotopes), with cyclotron-produced ^{18}F-fluoride ions at 96° C to synthesize ^{18}F-mefway. Reverse-phase C18 HPLC purification with a mobile phase of 50:50:0.1 MeCN:H_2O:TEA was then performed. Solvents were removed via C18 sep-pak extraction. The final product was formulated in 9 mL sterile saline and 1 mL EtOH, and filter sterilized.

^{18}F-Mefway PET data were acquired on a Siemens EXACT HR+ PET scanner using 3-D mode. A six minute transmission scan using ^{68}Ge rod sources was first acquired for attenuation correction. Dynamic PET data acquisition was initiated with a bolus injection of 192–204 MBq ^{18}F-mefway, and data were acquired for 120 minutes. Venous samples of 1.0 mL were acquired from the cephalic vein (opposite the injection arm) approximately 5, 15,

30, 60, 90, and 120 minutes postinjection. At least two weeks after the first scan, five of the six subjects returned for a second ^{18}F-mefway scanning procedure to assess the test-retest reproducibility of ^{18}F-mefway imaging. The mean and standard deviation of the administered mass of ^{18}F-mefway was 56±50 ng (range, 12–143 ng). The mean administered activity of ^{18}F-mefway was 199±4 MBq (range, 192–204 MBq). There were no adverse or clinically detectable pharmacologic effects, including no significant changes to vital signs or laboratory results, in any of the 6 subjects.

MRI data were acquired on a GE 3.0 T MR750 (Waukesha, WI) with an 8 channel head coil. A T_1-weighted SPGR volume was acquired using the following parameters: TI/TE/TR=450ms/3.2ms/8.2ms, flip angle=12°, slice thickness=1mm no gap, FOV=256, matrix size=256×256.

Data Processing and Analysis

Venous blood samples were analyzed to assay ^{18}F-mefway metabolism in vivo. Samples of 1.0 mL whole blood mixed with 50 μL heparinized saline were centrifuged for 5 minutes. Next, 0.5 mL plasma was extracted and mixed with 50 μL 5.5% sodium bicarbonate and 0.5 mL acetonitrile, followed by vigorous mixing to denature the proteins. After the protein precipitate settled, 0.5 mL liquid was extracted, concentrated, and spotted on an alumina TLC plate (Whatman). The TLC was developed in a mobile phase of 50:50 MeOH:10% ammonium acetate and subsequently exposed to a phosphor plate to quantify parent ^{18}F-mefway present in the blood. The phosphor plate was read by a Cyclone storage phosphor system (PerkinElmer) and analyzed with OptiQuant software. The plasma free fraction (f_P) was measured with Centrifree ultrafiltration units (Millipore).

PET data were histogrammed into frames of 8×0.5 minutes, 3×2 minutes, 10×5 minutes, and 6×10 minutes. Sinogram data were then reconstructed with a filtered back projection algorithm (Direct Inverse Fourier Transformation) using a 4 mm gaussian filter and included corrections for random events, deadtime, signal attenuation, and scanner normalization. Final images had dimensions of 128×128×63, corresponding to voxel dimensions of 2.57 mm × 2.57 mm × 2.43 mm. Images of PET data summed from 1–10 minutes postinjection, reflective of diffuse radioligand delivery throughout the brain, were used to register PET frames to the native space of the corresponding MRI image using FSL's Linear Image Registration Toolbox (FLIRT; 15). The affine matrix was constrained to a rigid body transformation (6 d.o.f.) since no intrasubject normalization was imposed.

Regions of interest were defined with FreeSurfer 5.3 software (http://surfer.nmr.mgh.harvard.edu). Regions extracted with this template-based algorithm included the amygdala, hippocampus, parahippocampal gyrus, insular cortex, anterior cingulate gyrus, posterior cingulate gyrus, parietal cortex, orbitofrontal cortex, temporal cortex, occipital cortex, and frontal cortex, shown in Figure 2. Additionally, the raphe nuclei region was manually drawn for each PET scan since this region's structure cannot be accurately determined based on MRI data. To observe radioligand kinetic properties in regions of highest ^{18}F-mefway binding, PET data from a manually defined region of focal uptake in the mesial temporal lobe were also analyzed. This region included areas of the hippocampus proper, subiculum, dentate gyrus, and amygdala, and was hand drawn to minimize imperfect

PET to MRI coregistration and partial volume effects. Time activity curves were extracted from all regions for subsequent analysis.

To compare measured cerebral radioactivity concentrations with other radioligands, standardized uptake value (SUV) was calculated as SUV = PET/i.d.* mass*1000, where PET is the measured PET concentration (kBq/cc), i.d. is the injected dose (kBq), and mass is subject mass (kg). To quantify specific ^{18}F-mefway binding, binding potential based on nondisplaceable uptake (BP_{ND}) was measured. BP_{ND} is an index of receptor binding proportional to the product of receptor density and radioligand-receptor affinity ($BP_{ND}=f_{ND}B_{max}/K_{Dapp}$; 16). Regional ^{18}F-mefway BP_{ND} values were calculated with the Multilinear Reference Tissue Model (MRTM; 17), assuming the cerebellum as a reference region. Logan graphical analysis (18) was used to calculate BP_{ND} values for comparison with the MRTM method and to visualize the establishment of radioligand psuedoequilbirum. Voxel-wise ^{18}F-mefway BP_{ND} maps were also produced with Logan graphical analysis.

To assess the test-retest reproducibility of ^{18}F-mefway binding, early PET data from the retest scans (1–10 minutes postinjection) were coregistered to the space of the test PET scan, allowing for 6 degrees of freedom. The affine matrix transforming the test PET data to the MRI was then applied to the retest PET data. TACs were extracted and regional BP_{ND} values using MRTM were calculated as described above. The test-retest variability (*TRV*) between test and retest BP_{ND} values expressed as a percentage was calculated for each region with the relationship $TRV=Abs\{(BP_{ND(Test)}-BP_{ND(Retest)})/(BP_{ND(Test)}+BP_{ND(Retest)})/2\}*100$. Additionally, the intraclass correlation coefficient (ICC; 19) was calculated for each region.

Results

Radiochemistry

^{18}F-Mefway was produced with high yields >1 GBq with specific activities >400 MBq/nmol. Radiochemical purity was 98.7±2.8%, with chemical purities and stability >90% at time of expiration.

^{18}F-Mefway in Plasma

Venous blood samples were acquired to characterize the in vivo metabolism of ^{18}F-mefway. Acceptable radiochromatograms were only acquired for four subjects. Radio-TLC analysis of the plasma samples revealed the presence of radiolabeled metabolites as shown in Figure 3, although no attempt was made to assess any volatile species. The chemical nature of these metabolites were not characterized in the present work. Notably, no radiolabeled species were detectable at the origin of the chromatogram, suggesting negligible accumulation of ^{18}F-fluoride ions in the blood.

In vivo metabolism of ^{18}F-mefway was initially rapid, with parent compound accounting for less than 20% of total plasma radioactivity 30 minutes postinjection. At this point, metabolism slowed, such that 10–15% of the radioactivity in the plasma was ^{18}F-mefway at

90 minutes postinjection (see Figure 3). The f_P of ^{18}F-mefway, measured for five of the six subjects, was 5.1 ± 0.7%.

^{18}F-Mefway Brain Uptake

Time activity curves of ^{18}F-mefway uptake in the brain are illustrated in Figure 4. The time course of ^{18}F-mefway in the brain rapidly peaked at SUVs of roughly 1.7 after 1–2 minutes in the cerebellum and various cortical regions. Clearance of ^{18}F-mefway was rapid in the cerebellum, decreasing to half the peak value within 10 minutes postinjection and approaching SUVs of 0.2 at 120 minutes postinjection. In regions of high specific ^{18}F-mefway uptake, such as the mesial temporal lobe and hippocampus, peak uptake of ^{18}F-mefway was slower, plateauing within 15–20 minutes postinjection with very slow decreases in PET signal, reflective of specific ^{18}F-mefway binding. As illustrated in Figure 5A, ratios of ^{18}F-mefway concentrations in hippocampal regions relative to the cerebellum plateaued after roughly 60–90 minutes postinjection at ratios ranging from 2 to 4.5. In cortical regions, these ratios plateaued faster with lower peak ratio values.

Specific ^{18}F-Mefway Binding

Estimates of BP_{ND} generated with MRTM2 for all regions examined are presented in Table 1. The highest values were observed in the MTL (2.42 ± 0.46). Estimates of BP_{ND} generated with the Logan reference region analysis method, using a linearization time t^*=45 minutes and omitting the k_2 term, agreed within 3% of the estimates with MRTM. Sample Logan plots are illustrated in Figure 5B, showing the linearization of the data in all regions by 45 minutes postinjection. A voxelwise BP_{ND} map, generated with Logan graphical analysis, is shown in Figure 6 for visualization of ^{18}F-mefway specific binding.

Retest ^{18}F-mefway scans were acquired for five subjects, however, one subject did not complete the scanning procedure, therefore only four retest scans were analyzed for test-retest analysis. For the four subjects with retest scans, the TRV averaged across all regions was 8% (Table 1). The ICC in regions of high ^{18}F-mefway uptake was greater than 0.88, indicating substantial agreement (20). Lower values were measured in areas of moderate uptake, likely due to the reduced BP_{ND} values in these regions.

Discussion

This study demonstrates that ^{18}F-mefway has favorable specific binding levels and kinetic properties favorable for human PET imaging of 5-HT_{1A} receptors. Reliable imaging of the 5-HT_{1A} system has been an important goal of the neuroimaging community, yet lack of a suitable radioligand has stunted successful widespread PET imaging of 5-HT_{1A} receptors due to limitations in areas such as radiochemistry, quantitation, defluorination, and brain penetration. ^{18}F-Mefway possesses a simple radiochemical production, brain uptake levels comparable to other 5-HT_{1A} radioligands, high signal to noise ratio, suitable kinetic properties, no apparent PET signal in bone, and higher detected nonspecific PET signal compared to ^{11}C radioligands, making it a promising candidate for imaging 5-HT_{1A} receptors in humans.

Radio-TLC techniques were used to measure the rate of ^{18}F-mefway metabolism in vivo. The results indicated initial rapid metabolism of ^{18}F-mefway followed by a slow metabolism component such that 10–15% of the radioactivity in the plasma was attributed to parent ^{18}F-mefway after 90 minutes in the blood. Low counting statistics in the blood samples at late times due to rapid metabolism and low f_P limited the precision of these measurements. The rate of ^{18}F-mefway metabolism was slightly slower than ^{11}C-WAY-100635 and ^{18}F-FCWAY (21, 22).

The in vivo metabolism of ^{11}C-WAY-100635 and ^{18}F-FCWAY result in radiolabeled cyclohexanecaryboxylic acid species, both of which crossed the blood brain barrier (21, 22). A similar potential metabolite species of ^{18}F-mefway, ^{18}F-*trans*-4-fluoromethylcyclohexanoic acid, showed little to no brain penetration in rat PET studies (23). We have not fully characterized the metabolite species of ^{18}F-mefway in humans for the present work, thus the potential for ^{18}F-*trans*-4-fluoromethylcyclohexanoic acid accumulation in human brain remains a matter for future studies. The f_P of ^{18}F-mefway was measured at 5.1%, with little variability between subjects. This low free fraction is consistent with the values of other radioligands specific to 5-HT$_{1A}$ receptors.

Specific uptake of ^{18}F-mefway in human brain was consistent with the cerebral distribution of 5-HT$_{1A}$ receptors (24). The highest measured ^{18}F-mefway BP_{ND} levels were 2.4 in the regions in the mesial temporal lobe, with values of 1.6 in insular cortex, 1.2 in anterior cingulate gyrus, 0.8 in raphe nuclei, and 0.6–0.9 in occipital cortex. These human ^{18}F-mefway BP_{ND} values are roughly 3–4 times lower than reported ^{11}C-WAY-100635 BP_{ND} values derived using an atlas based approach (25). However, a direct comparison of these radioligands in human subjects with the same scanners and data processing techniques will be needed to identify in vivo differences between radiotracers. Such studies previously performed in nonhuman primates demonstrated comparable levels of BP_{ND} between ^{18}F-mefway and ^{11}C-WAY-100635 (11). While interspecies differences may explain some of the variation in ^{18}F-mefway binding between humans and monkeys, the atlas-based ROI definition in this work likely reduced BP_{ND} due to spatial averaging compared to the manual ROI definition in our previous work.

The behavior of 5-HT$_{1A}$-specific radioligands in the cerebellum is a crucial issue for accurate assay of 5-HT$_{1A}$ binding. Use of the cerebellum with reference region analysis strategies can avoid the need for arterial blood sampling. The cerebellum has been used as a reference region for quantitation of BP_{ND} with 5-HT$_{1A}$ radioligands due to minimal specific binding levels (26). Small levels of specific ^{11}C-WAY-100635 binding were subsequently observed in the cerebellar gray matter and vermis (27), indicating a potential underestimation in BP_{ND} with the use of cerebellar gray matter. White matter regions have been proposed as potential reference regions to avoid this bias of BP_{ND} estimates (28,29), which assumes similar nonspecific radioligand behavior in both white matter and gray matter. We speculate that the strategies developed to account for potential binding of other 5-HT$_{1A}$ radioligands in the cerebellum will be applicable to ^{18}F-mefway procedures as well. Further investigation is needed to fully characterize potential specific binding in the cerebellum and its ramifications on the analysis of ^{18}F-mefway PET data.

The time course of ^{18}F-mefway in the cerebellum was characterized by rapid washout followed by low radioactivity concentrations (Figure 4). These cerebellar characteristics are similar for other 5-HT$_{1A}$ radioligands, resulting in low counting statistics in the cerebellum at late times. Such regions are subject to bias depending on the scatter correction and reconstruction algorithms used in processing the PET data (30), and subsequently bias BP_{ND} estimates when used as reference regions. Given the similar cerebellar SUV values of ^{18}F-mefway and ^{11}C-WAY-10036 (31) at 90 minutes, the 110 minute half-life of the ^{18}F isotope compared to the 20 minute half-life of ^{11}C yields over 12-fold more real measured counts by the PET scanner. This characteristic, and the opportunity to conduct PET studies with an off-site cyclotron, are important practical advantages of ^{18}F-labelled 5-HT$_{1A}$ radioligands. One potential cause of low cerebellar uptake for 5-HT$_{1A}$ specific radioligands is the p-glycoprotein transporter, as demonstrated for ^{18}F-MPPF (32,33). The potential regulation of ^{18}F-mefway brain penetration by p-glycoprotein is an important question for future investigation.

Previous ^{18}F-FCWAY studies exhibited bone uptake of ^{18}F-fluoride ions, resulting in the spill-in of detected PET signal from the surrounding skull into the cerebellum and subsequently required the use of enzyme inhibitors for accurate ^{18}F-FCWAY quantification (7, 8). Low levels of PET signal in bone were evident in rat ^{18}F-mefway studies (34), but not in rhesus monkeys (11). The present data were closely inspected for potential bone uptake of radiolabeled species in human subjects. The PET images did not indicate any areas of elevated ^{18}F-mefway uptake in regions immediately surrounding the brain. Cerebellar time activity curves decreased at late times, instead of plateauing or increasing (which would reflect the spill-in of PET signal from surrounding bone). Furthermore, the radio-TLC analysis did not reveal a detectable signal at the expected location of ^{18}F-fluoride ion elution (the origin; Figure 3A). These studies provide evidence for negligible accumulation of radiolabeled species in bone, however, they are not conclusive. Future planned PET-CT studies, providing accurate localization of skull, will conclusively determine the reported absence of bone uptake. Typical ^{11}C-WAY-100635 scans require 90 minutes for accurate quantification. While 120 minutes of ^{18}F-mefway data were acquired for the present scans, the data were truncated at 90 minutes and BP_{ND} values were recalculated with MRTM. Calculated BP_{ND} from the 90 minutes truncated data set agreed well with BP_{ND} from the full data set, with R^2=0.99. There was a slight negative bias due to a systematic BP_{ND} underestimation of 3% in the mesial temporal lobe regions of highest ^{18}F-mefway binding. This small bias in regions of extreme ^{18}F-mefway binding may be acceptable in exchange for reduced duration of scanning procedures. Therefore it is likely that 90 minutes will be an appropriate scanning time for accurate quantification of ^{18}F-mefway binding.

Values of ^{18}F-mefway TRV averaged 8% across all regions, indicating good agreement in repeated scans. The ICC values were very strong in regions of high ^{18}F-mefway binding. Reduced ICC values were observed in regions of lower ^{18}F-mefway specific binding, likely due to lower BP_{ND} values in these regions. The test-retest properties of ^{18}F-mefway are encouraging for future human PET studies implementing a two-scan experimental design.

The binding profile of ^{18}F-mefway allowed for close inspection of the regional distribution of specific binding in the mesial temporal lobe, as visualized in Figure 7. The region of

highest ^{18}F-mefway binding was the hippocampus. Relatively lower binding levels were observed in the subiculum sublayer, located more medially than CA1-CA4, and the amygdala. Less ^{18}F-mefway binding was also evident in the parahippocampal gyrus and the most posterior regions of the hippocampus near the crux of the fornix. These differences in specific ^{18}F-mefway uptake yielded exquisite delineation of 5-HT$_{1A}$ binding in the mesial temporal lobe. Consequently, ^{18}F-mefway PET data from this region, most prominently in the hippocampus, may have clinical utility in studying both healthy pathology and neurological and psychiatric disorders that affect the mesial temporal lobe.

Conclusion

These initial studies of ^{18}F-mefway in humans prove highly promising. The simple radiochemical production, high specific radioligand uptake, ^{18}F radiolabel, and lack of PET signal in bone make ^{18}F-mefway a promising candidate for assay of 5-HT$_{1A}$ receptors with human PET. Future studies to assess the viability of ^{18}F-mefway in pathology-specific populations are merited.

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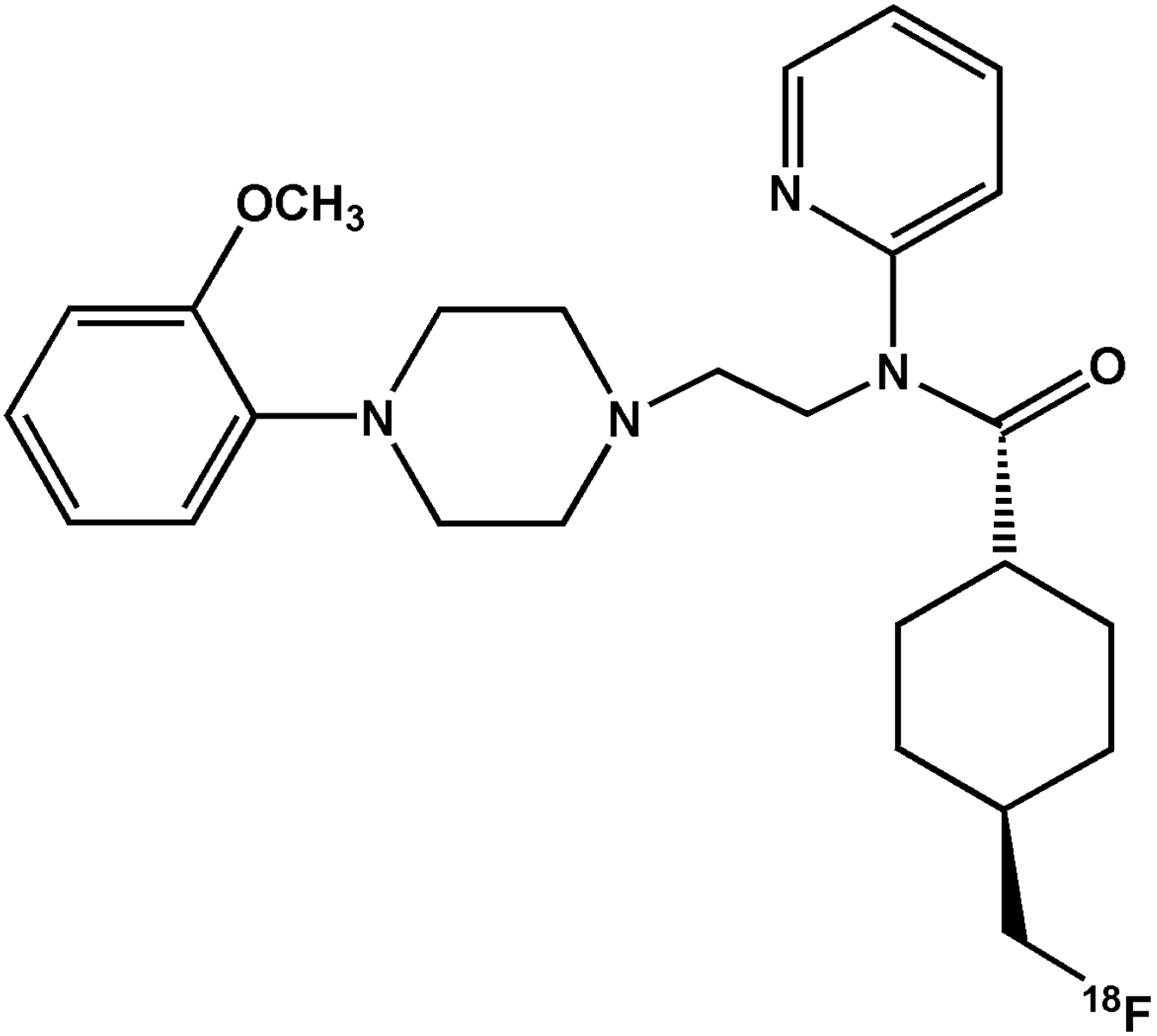

Figure 1.
Chemical structure of *trans*-^{18}F-Mefway.

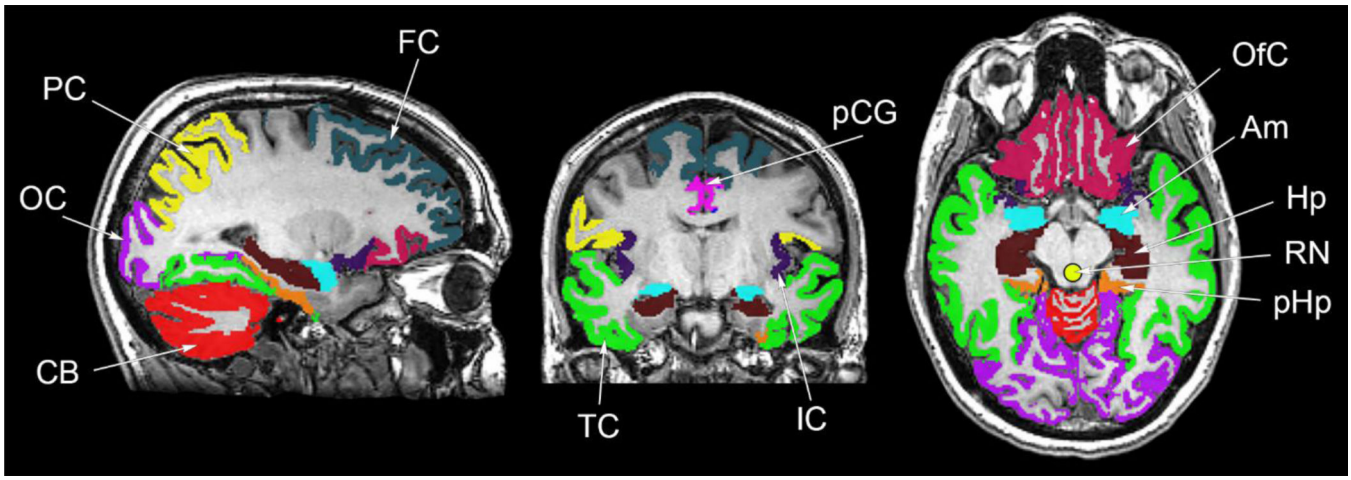

Figure 2.

Regions used for analysis of ^{18}F-mefway PET data. Regions defined by the template-based FreeSurfer algorithm included the amygdala (Am), hippocampus (Hp), parahippocampal gyrus (pHp), insular cortex (IC), anterior cingulate gyrus (aCG; not shown), posterior cingulate gyrus (pCG), parietal cortex (PC) orbitofrontal cortex (OfC), temporal cortex (TC), occipital cortex (OC), frontal cortex (FC). The hand drawn raphe nuclei (RN) is also shown.

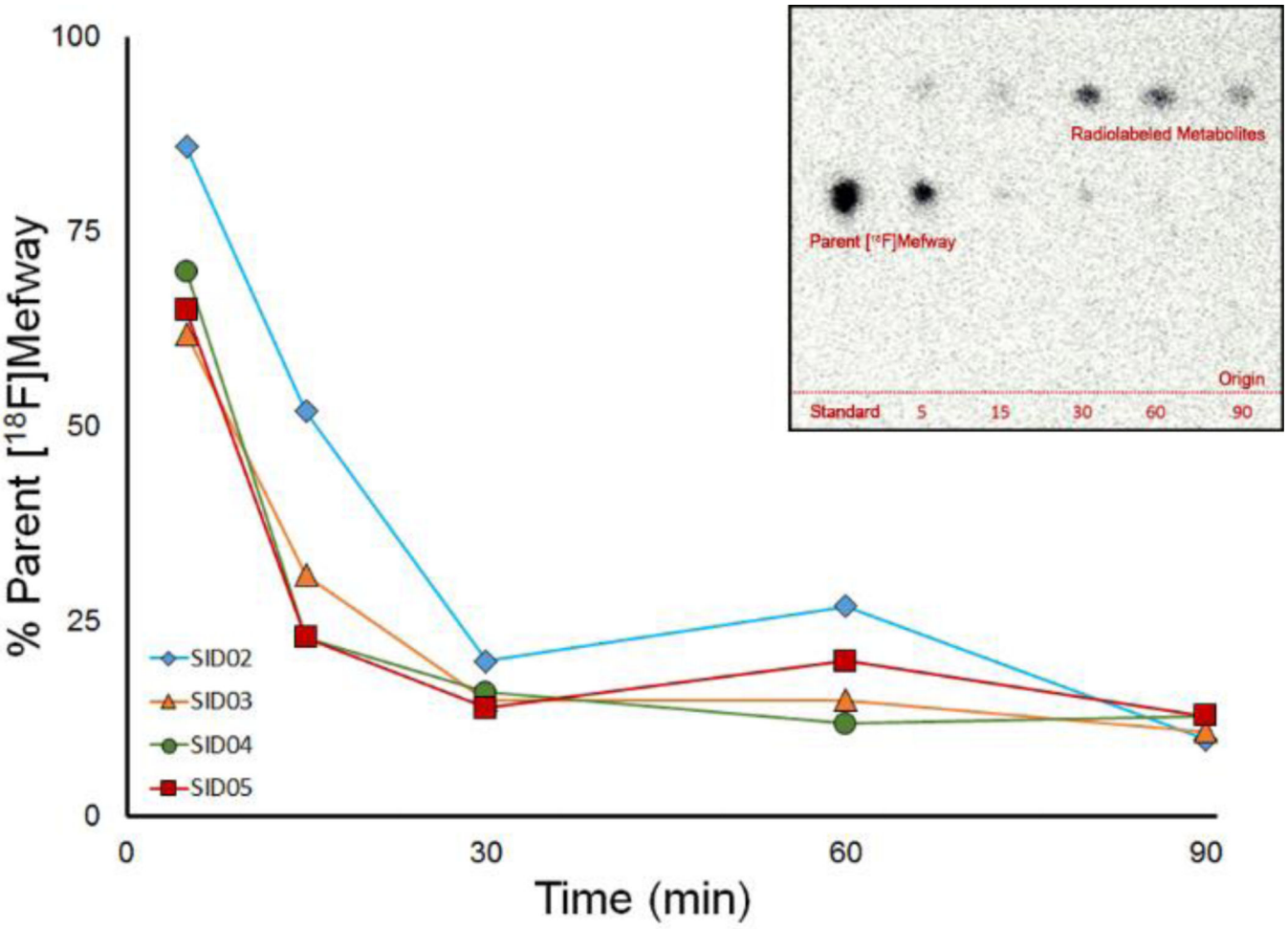

Figure 3.

Metabolism of ^{18}F-mefway *in vivo*. The main plot shows the percentage of total radioactivity in the plasma attributed to parent ^{18}F-mefway. Each distinct color and shape corresponds to a separate subject. The inset presents a typical radiochromatogram. Note the absence of detectable radioactivity at the origin, the expected location of ^{18}F-fluoride ion elution.

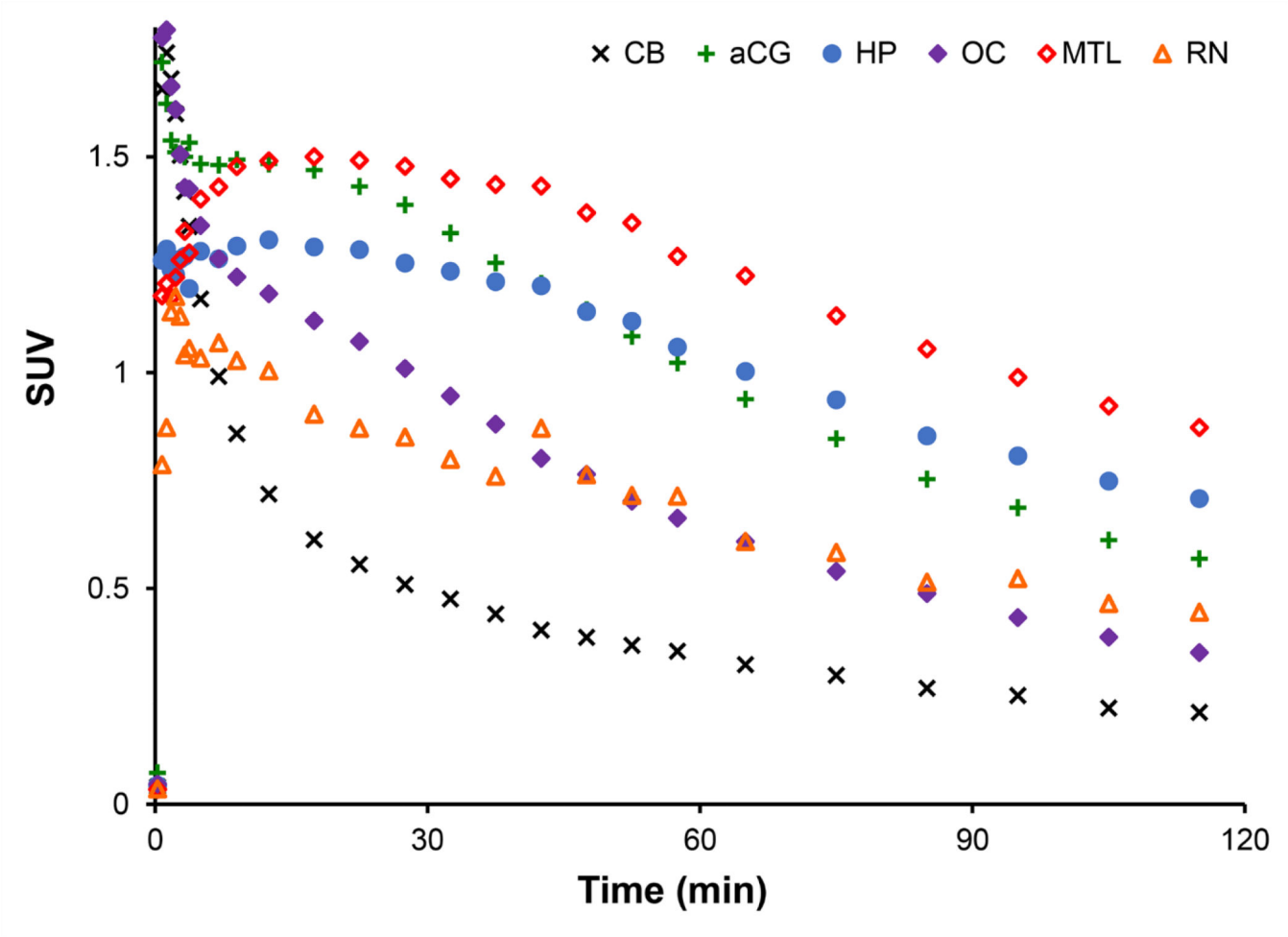

Figure 4.

Representative ^{18}F-mefway time-activity curves. SUVs are defined as SUV = PET/i.d.* weight*1000. Regions shown include focal areas of uptake in the mesial temporal lobe (MTL, ◊); hippocampus (Hp, ●); anterior cingulate gyrus (aCG, +); raphe nuclei (RN, △); occipital cortex (OC, ♦); and cerebellum (CB, ×).

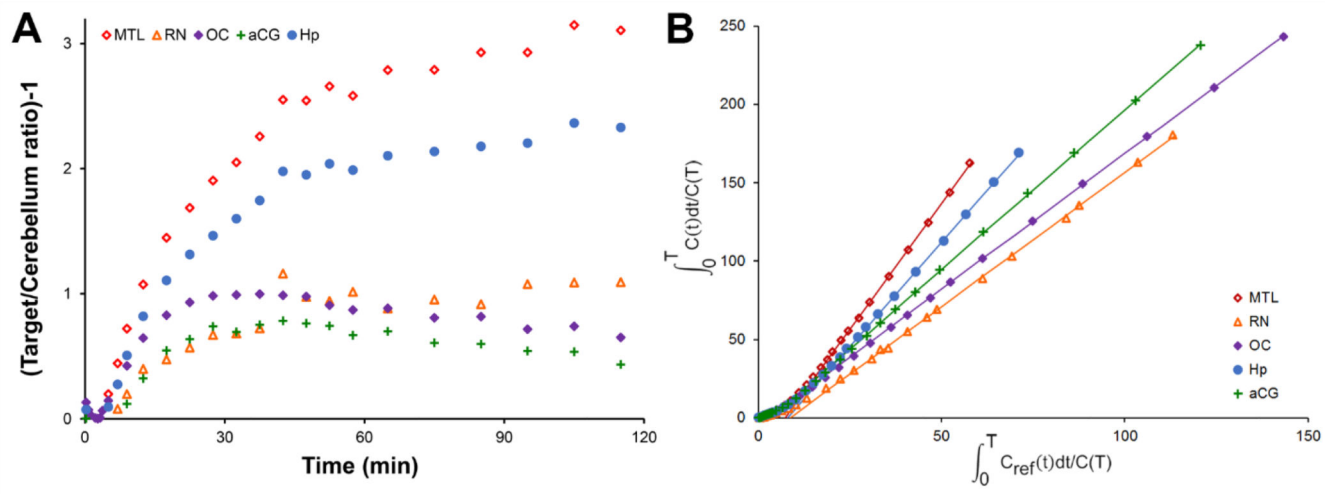

Figure 5.

Kinetic properties of ^{18}F-mefway *in vivo*. **A** shows ^{18}F-mefway target/cerebellum ratios. **B** illustrates Logan plots, with t^{*}=45 minutes, to visualize pseudoequilibrium of the tracer in various regions of the brain. Regions shown include focal areas of uptake in the mesial temporal lobe (MTL, ◊); hippocampus (Hp, +); anterior cingulate gyrus (aCG, ●); raphe nuclei (RN, Δ); and occipital cortex (OC, ♦).

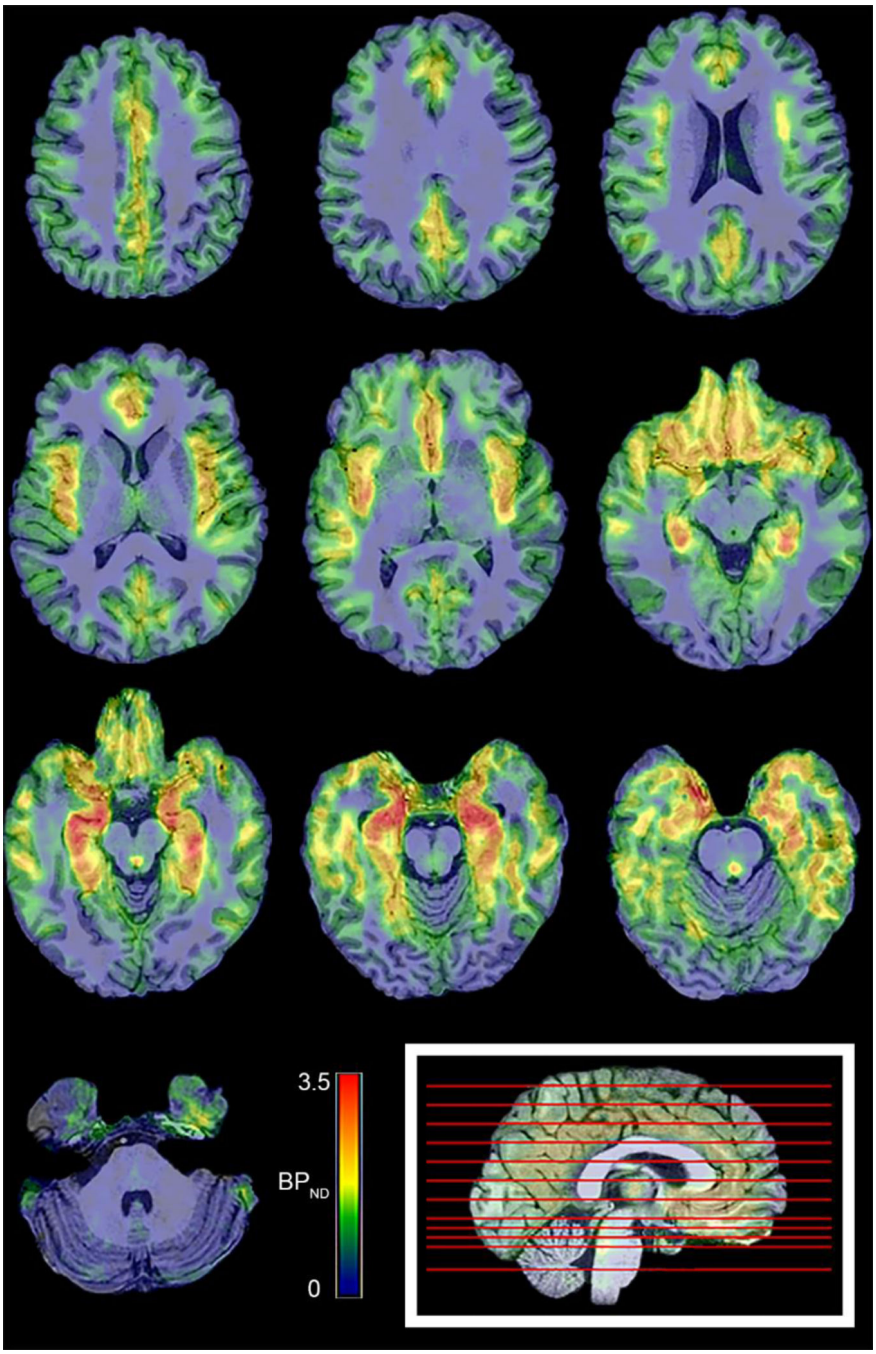

Figure 6.

Voxel-Wise ^{18}F-mefway BP_{ND} maps overlaid on a coregistered MRI image. Parametric BP_{ND} images are linearly scaled from 0 to 3.5. The location of each transverse slice is denoted by red lines on the mid-sagittal slice at bottom right.

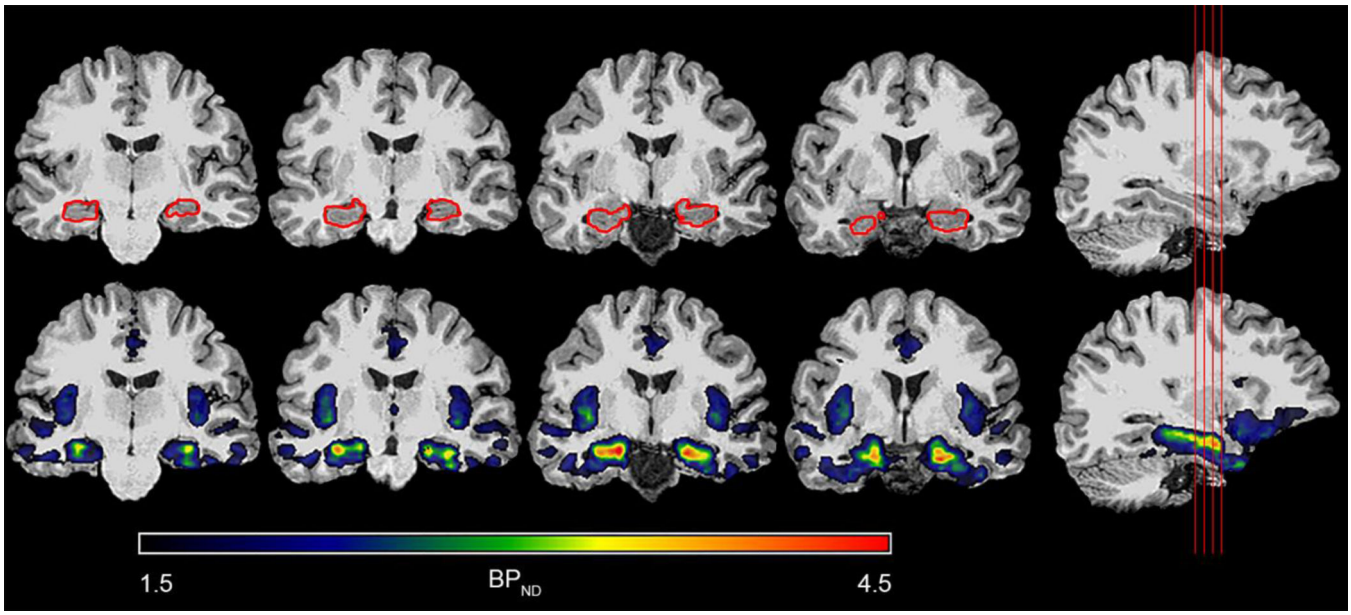

Figure 7.

Delineation of specific ^{18}F-mefway binding in the mesial temporal lobe. The top row shows a T_1-weighted MRI with the corresponding hippocampal FreeSurfer mask drawn in red, while the bottom row shows a ^{18}F-mefway BP_{ND} parametric image overlaid on the same MRI. Note the BP_{ND} linear thresholding ranges from 1.5 to 4.5. The red lines on the far right sagittal slice indicate the location of the corresponding coronal slices.

Table 1

Scan details and regional ^{18}F-mefway BP_{ND} values with test-retest analysis

					Regional BP_{ND}#												
Subject	**Sex**	**Age**	**Weight (kg)**	**Injected Activity (MBq)**	**MTL***	**Hp**	**Am**	**pHp**	**IC**	**aCG**	**pCG**	**RN***	**PC**	**OfC**	**TC**	**OC**	**FC**
1	F	27	89	204	2.59	2.08	1.14	1.74	1.61	1.08	0.86	0.94	0.73	1.08	1.27	0.63	0.77
2	F	27	79	192	2.23	1.67	1.12	1.65	1.53	1.07	0.73	0.72	0.95	0.88	1.39	0.75	0.99
3	M	32	113	200	3.15	2.65	2.08	2.24	2.19	1.83	1.54	1.56	0.80	1.68	1.31	0.79	0.81
4	M	22	97	204	1.86	1.14	0.52	1.27	1.27	0.71	0.51	0.37	0.87	0.85	1.27	0.64	0.79
5	F	38	114	196	2.63	1.85	1.03	1.80	1.40	1.13	0.85	0.80	0.72	1.02	1.20	0.66	0.73
6	F	38	58	196	2.07	1.11	0.72	1.10	1.52	1.50	0.87	0.59	1.27	1.31	1.51	0.89	1.37
Mean (S.D.)					**2.42 (0.46)**	**1.75 (0.59)**	**1.10 (0.54)**	**1.63 (0.41)**	**1.59 (0.32)**	**1.22 (0.39)**	**0.89 (0.34)**	**0.83 (0.41)**	**0.89 (0.20)**	**1.14 (0.31)**	**1.32 (0.11)**	**0.73 (0.10)**	**0.91 (0.24)**
Test-Retest Variability (TRV)					5.89	3.69	11.55	5.42	11.04	8.94	10.01	11.35	10.06	8.16	5.61	4.10	9.04
Intra-Class Correlation (ICC)					0.94	0.99	0.99	0.95	0.88	0.98	0.98	0.97	0.60	0.96	0.43	0.88	0.71

*Denotes PET defined regions

#BPND values calculated with MRTM.

Regions included are focal areas of uptake in the mesial temporal lobe (MTL; hand drawn), hippocampus (Hp), amygdala (Am), parahippocampal gyrus (pHp), insular cortex (IC), anterior cingulate gyrus (aCG), posterior cingulate gyrus (pCG), parietal cortex (PC) orbitofrontal cortex (OfC), temporal cortex (TC), occipital cortex (OC), frontal cortex (FC), and raphe nuclei (RN; PET defined).