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Which is the Best Threshold for Asymmetry Indices for Single Subjects 5-ht1a Receptor Pet Studies in Temporal Lobe Epilepsy? a Discriminant Analysis Validation

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WHICH IS THE BEST THRESHOLD FOR ASYMMETRY INDICES FOR SINGLE SUBJECTS 5-HT$_{1A}$ RECEPTOR PET STUDIES IN TEMPORAL LOBE EPILEPSY? A DISCRIMINANT ANALYSIS VALIDATION

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Running head: 5-HT$_{1A}$ receptor discriminant analysis in epilepsy

ABSTRACT

For identification of the epileptogenic focus in single subject PET studies of temporal lobe epilepsy patients (TLE) asymmetry indices (AIs) are commonly used, but their use is not validated. We assessed the accuracy of different AIs thresholds to discriminate between TLE patients and healthy controls using linear discriminant analysis. Twenty-two TLE patients and ten controls were studied with the 5-HT_{1A} receptor ligand [^{18}F]FCWAY. An automated, MR-segmentation-based partial volume correction (PVC) algorithm was applied. Significant asymmetries were defined, respectively, as greater than control mean AI + 1 SD (cut-off 1) and greater than control mean AI + 2 SDs (cut-off 2). The differences (residuals) between patient AIs and cut-offs were calculated for each region and used as input to a stepwise linear discriminant analysis. Leave-one-out cross validation was applied. Before PVC, correct classification rates were, respectively 91% and 100% using cut-off 1 and cut-off 2. After PVC, correct classification rates were, respectively 97% and 100% using cut-off 1 and cut-off 2. AIs in the hippocampus and in the occipital cortex were selected as significant predictors in all analyses. Control mean plus 2 SDs is the best threshold to identify with good accuracy asymmetries in 5-HT_{1A} receptor PET images of TLE patients.

INTRODUCTION

Serotonin (5-HT) 5-HT_{1A} receptor positron emission tomography (PET) ligands are valuable, together with [^{18}F]-fluorodeoxy-2-glucose (FDG) and [^{11}C]flumazenil, for the presurgical evaluation of drug-resistant temporal lobe epilepsy (TLE). Research studies typically report unpaired group comparisons with normal subjects. Numerous PET studies in TLE found reduction of 5-HT_{1A} receptor specific binding in several temporal regions. These differences are more numerous in the side ipsilateral, rather than contralateral, to the seizure focus, and more prominent for mesial, rather than lateral, temporal regions, and in the raphe [1-7].

In clinical practice there is a need to read single subject PET scans. As in other neuropsychiatric disorders, where visual reading of brain PET images is often supplemented by a semiquantitative index, asymmetry indices (AIs) of single subjects' PET have been used for many decades in epilepsy. The use of AIs is based on observations of reduced tracer uptake in brain structures ipsilateral to the epileptogenic focus, demonstrated with all main tracers used in TLE, including FDG [8, 9], [^{11}C]flumazenil [10, 11] and 5-HT_{1A} receptor ligands [1-7]. However, for single patient analysis AI thresholds have varied depending on the laboratory and tracer, and application of thresholds has been based on 'clinical sense' but not formally validated.

Discriminant analysis is a statistical technique that can be used to examine whether two mutually exclusive groups can be distinguished from each other based on a linear combination of values of predictor variables. Identification of a discriminant function assumes existence of a disease-specific pattern, such as side-to-side asymmetry related to the epileptogenic focus, as well as the absence of side-to-side differences in unaffected areas.

We reported previously that reductions of 5-HT_{1A} receptors specific binding in TLE patients compared to healthy controls in mesial, but not lateral, temporal structures and in the

insula remained highly significant after partial volume correction (PVC) [5]. The present study is a re-analysis of 5-HT_{1A} receptor concentration of TLE patients and healthy controls included in this previous study [5]. The aim of the current study was to find out the best threshold for identification of significant asymmetries in 5-HT_{1A} receptor concentration by comparing the classification of healthy controls and TLE patients using linear discriminant analysis derived by two different asymmetry thresholds.

MATERIAL AND METHODS

Subject selection. Twenty-two patients (18 males; mean age ± standard deviation (SD) was 37 ± 11 years) and ten healthy volunteers (7 males; 35 ± 9 yrs) were recruited by the Clinical Epilepsy Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health (NIH). Subjects were studied under a clinical protocol approved by the Institutional Review Board of the National Institute on Neurological Disorders and Stroke and the Radiation Safety Committee, NIH. The clinical correlations of electroencephalography (EEG), magnetic resonance, and 5-HT_{1A} [^{18}F]FCWAY binding were previously reported [5]. Seizure onset had been localized with ictal video EEG with scalp electrodes and intracranial subdural electrode monitoring, as indicated. None of the patients experienced a seizure for at least 2 days prior to PET scan and none had a secondarily generalized seizure for at least 1 month. Written informed consent according to the Declaration of Helsinki was obtained from all subjects.

Magnetic resonance (MR) procedure. All subjects underwent MR imaging using a 1.5 or a 3.0 Tesla Horizon (General Electric, Waukesha, WI). In all subjects, fluid attenuated inversion recovery was acquired for the evaluation of mesial temporal sclerosis. In patients, 3D T1-weighted volumetric spoiled gradient recovery (SPGR) coronal MR images, and in healthy subjects 3D T1-weighted transverse SPGR images were acquired. Both MR images had the same

spatial resolution (voxel size, 0.94 x 0.94 x 1.5 mm; 256 x 256 x 124 slices). Transverse slices were resliced to the coronal plane for all analyses. Standard coronal T2 and FLAIR (Fluid Attenuated Inversion Recovery) images were obtained.

Radiochemistry. [^{18}F]FCWAY was synthesized as described by Lang et al [12]. Specific activity at time of injection was greater than 500 Ci/mmol (1600 ± 1100, n=23).

PET procedure. Each subject was scanned with a General Electric (Waukesha, WI, USA) Advance Tomograph (FWHM = 6-7 mm). Scans were obtained in a quiet, dim room as previously described [5]. Continuous EEG monitoring was performed to ensure an interictal study. Blood samples were collected from the subject's radial artery throughout the PET scan, and radioactivity in whole blood and plasma was determined. To correct for attenuation, a transmission scan was performed. Subsequently, a bolus of 9 ± 2 mCi of [^{18}F]FCWAY was injected over 60 seconds and dynamic scanning was performed in 3D-mode for a period of 120 minutes with 33 frames of increasing length (number x frame duration in minutes: 6 x 0.5; 3 x 1; 2 x 2; 22 x 5). Images were corrected for randoms and scatter, and reconstructed using an iterative algorithm.

[^{18}F]FCWAY input function. About 30 radial arterial samples were taken to quantify [^{18}F]FCWAY concentration and selected samples used to measure the [^{18}F]-fluorocyclohexanecarboxylic acid metabolite [13]. Unbound [^{18}F]FCWAY fraction plasma protein was measured with ultracentrifugation [14].

Data analysis. Time activity curves (TACs) for plasma and whole blood were obtained with linear interpolation. Linear interpolation was felt an adequate strategy because the time-dependent frequency of the arterial sampling allowed good temporal resolution of the blood curve. [^{18}F]FC plasma concentrations were fitted to a multiexponential function. [^{18}F]FCWAY time activity curves were corrected using the measured [^{18}F]FC input function, a 1-tissue compartment model and parameters obtained in monkeys [13, 15]. Correction for vascular

radioactivity was performed assuming a 5% blood volume. Corrected PET frames were registered to MR images using a mutual information algorithm [16]. TACs were fitted pixel-by-pixel to a reversible two-tissue compartment model using metabolite-corrected input function. In performing the fit, pixel data were weighted by the inverse of variance, estimated from total prompt and delayed coincidences, scatter fraction, decay, and scan duration. The model equation is:

$$([A_1 \exp(-\alpha_1 t) + A_2 \exp(-\alpha_2 t)] \otimes C_p(t))$$

with 3 floating parameters (A_1, α_1, and A_2) and one fixed parameter (α_2) from which parametric images of K_1 and V_T are derived [15]. The fast component of the bi-exponential function (α_2) was fixed at the mean value (0.32 min^{-1}) obtained from about 100 ROIs in normal controls, after exclusion of the 20% of ROIs with unstable 2T fits [15]. This model has only one non-linear parameter (α_1) allowing rapid imaging calculation [15]. In a two-tissue compartment model, the total distribution volume (V_T) equals [17, 18]:

$$V_T = K_1/k_2 \cdot (1 + k_3/k_4).$$

Partial volume correction (PVC) algorithm. The algorithm [19] provides voxel-by-voxel binary masks of gray matter, white matter, and CSF. Gray matter pixels are corrected for spill-out of gray matter activity, as well as for spill-in of activity from white matter. CSF activity is set to zero. Brain white matter activity is assumed to be uniform [20]. Subsequently, the binary masks are smoothed to the PET resolution by convolving the binary mask with a 6-mm FWHM 3-dimensional Gaussian kernel (based on the reconstructed image resolution); these masks are named as s_{GM}, s_{WM}, and s_{CSF}. The corrected activity gray matter values can be calculated as follows:

$$C_{PVC} = \frac{C_{ORIG} - C_{WM}\, s_{WM}}{s_{GM}}$$

where C_{PVC} represents the corrected activity value in a gray matter pixel after PVC, C_{ORIG} is the original uncorrected pixel value, C_{WM} is the estimated white matter activity [21, 22], and s_{GM} and s_{WM} are the pixel values from the smoothed masks for gray and white matter, respectively.

Region of interest analysis (ROI) analysis. Cerebellar white matter was used to estimate 5-HT_{1A} receptor free plus nonspecific binding, as previously described [5, 23]. To avoid spillover of [^{18}F]fluoride bone activity, cerebellar ROIs were placed far from the cortical edge. When needed, also cortical ROIs were manually adjusted and moved away from the cortical edge as necessary [5, 23]. 3D hippocampal ROIs were manually drawn on MR volumes using an anatomic atlas [24]. Large regions of interest ensured gray matter sampling and increased signal-to-noise ratio. Due to higher resolution of MR than PET data, ROIs were placed on 6 or 7 consecutive slices for amygdala, every second or third slice for hippocampus, fusiform, and parahippocampus gyrus, and every third or fourth slice for remaining cortical regions and cerebellum [5].

In the cortex, ROI measurements were computed using only gray matter pixels, as defined by the gray matter segment [19]. For cerebellum, where gray matter segment definition is not accurate, mean ROI value was computed from unmasked PET images coregistered to MR volumes. PET measurements were not performed for thalamus and basal ganglia, where segmentation was poor, and for raphe, which is an unpaired region.

The receptor binding outcome was $BP_F = [(V_T - V_{ND})/f_P)]$ [25], where V_T is the regional total distribution volume and V_{ND} is the distribution volume in cerebellar white matter, which is assumed to be devoid of specific binding [26].

Asymmetry indices (AIs), expressed in percentage, were computed as 200*(C – I)/(C + I), where C and I represent the BP_F values in the region contralateral and ipsilateral to the seizure focus, respectively.

Linear discriminant analysis. To derive the discriminant function, the following procedure was adopted. First, for the control group, normative thresholds were derived for each ROI. For each region the values of the mean AI + 1 SD (cut-off 1) and mean AI + 2 SDs (cut-off 2) were calculated. Then, for each patient the residuals (differences) between the AI in each region and cut-off 1 and cut-off 2 were calculated obtaining, respectively, residuals_1 and residuals_2. These residuals were given as input to the discriminant analysis. Discriminant analysis requires two groups each with a certain variable value. Since residuals, as described here, can be calculated only for TLE patients, the original AIs were given as input to the discriminant analysis for the healthy control group. The discriminant analysis searches for the linear combination of residuals that allows best discrimination between healthy controls and TLE patients. All regions were entered to the discriminant analysis using a stepwise method (F to enter, $P < 0.05$, F to remove, $P > 0.10$). Cross validation was performed with the leave-one-out procedure. Regional unstandardized discriminant function coefficients were used to compute the group membership probability. Statistical significance was taken at $P < 0.05$. Data analysis was performed using SPSS 31 (SPSS Inc, Chicago, IL, USA).

RESULTS

The numbers of patients with AI greater than cut-off 1 and cut-off 2 for each region before and after PVC are displayed in Table 1. As expected, the number of asymmetries decreased with cut-off 2 compared to cut-off 1 both before and after PVC (paired t-test: $P < 0.01$ in both cases). In all conditions, asymmetries were maximal in hippocampus and minimal in the occipital lobe.

The results of the discriminant analysis are shown in Table 2. Results were obtained using all ROIs in each subject. Before PVC, using cut-off 1, AIs in hippocampus and occipital cortex were selected as significant predictors. All controls and 19 patients were correctly classified to

their respective membership group (total correct classification rate = 91%). Using cut-off 2, AIs in hippocamps, inferior temporal cortex and occipital cortex were selected as significant predictors. All controls and all patients were correctly classified to their respective membership group (total correct classification rate = 100%).

After PVC, using cut-off 1, AIs in hippocampus, insula and occipital cortex were selected as significant predictors. All controls and 21 TLE patients were correctly classified to their respective membership group (total correct classification rate = 97%). Using cut-off 2, AIs in hippocampus, insula and occipital cortex were selected as significant predictors. All controls and all patients were correctly classified to their respective membership group (total correct classification rate = 100%).

DISCUSSION

To the best of our knowledge, this is the first application of linear discriminant analysis to 5-HT_{1A} receptor PET studies in TLE patients. We applied this technique to the 5-HT_{1A} ligand [^{18}F]FCWAY to identify the best threshold of AIs to distinguish TLE patients from healthy controls in single subject analysis.

Total correct classification rates were high with both cut-off 1 and cut-off 2, ranging from 91% to 100%. Correct classification rates were maximal for cut-off 2 (100%) both before and after PVC, while for cut-off 1 they were slightly lower than for cut-off 2 and slightly higher for PVC-corrected data. Based on these findings, reliable identification of the epileptogenic area can be performed using the 5-HT_{1A} receptor ligand [^{18}F]FCWAY using cut-off 2.

There is necessity to have accurate evaluation of single subjects 5-HT_{1A} receptor PET images in the presurgical evaluation of TLE patients. Single subjects findings have been previously related to clinical features, intracerebral EEG recordings, MR findings, and surgical

outcome [1, 2, 6, 27]. Thus, this study represents the ultimate final step in the attempt to validate single subject 5-HT_{1A} receptor PET studies.

Identification of the ideal cut-off must meet the compromise between sensitivity and specificity. For presurgical evaluation of TLE patients both parameters are important. [^{18}F]FCWAY has relatively large variability in healthy subjects. No healthy subject was misclassified as TLE patient (no false positives) showing that very high specificity can be obtained even with a threshold as low as cut-off 1. Using uncorrected images, three false negatives were detected with cut-off 1 and none with cut-off 2. The higher number of false negatives when using cut-off 1 as compared to cut-off 2 may appear contradictory because a higher threshold (e.g., cut-off 2) is expected to be associated with higher specificity but less sensitivity. However, note that the discriminant analysis does not directly compare AIs of controls and TLE patients, rather it searches, on a subject-by-subject basis, for the linear combination of residuals that maximizes the group differences. Moreover, in some TLE patients, reductions of the binding potential are bilateral [5], and therefore in such circumstances in the single patient AIs may be attenuated and a TLE patient can erroneously be misidentified as a healthy subject. Thus, increased sensitivity can occur with more restrictive cut-off criteria. The better performance of cut-off 2 compared to cut-off 1 has clinical implications because the correct subject identification obtained using cut-off 2 was associated with the correct EEG lateralization identified by ictal video EEG.

Even though results differed slightly accordingly to the analysis, two anatomical structures were selected as significant predictors in all analyses, i.e. hippocampus and occipital cortex. The involvement of hippocampus is consistent with the pathoanatomy of TLE, as indicated by animal models [28], by MR studies, reporting hippocampal atrophy and mesial temporal sclerosis (MTS) [29-32] by FDG studies, reporting hippocampal hypometabolism [33-37], and by

previous 5-HT_{1A} receptor studies, reporting reduction of the binding potential in this region [1-6].

The selection of occipital cortex might be an unexpected result given the fact that in this sample we previously reported no significant group differences in either BP_F or AIs in the occipital cortex, as well as in other extra-temporal regions [5]. Similar results were obtained by other groups [1-3]. However, the mechanism by which discriminant analysis identifies factors (that is, regions) to assign single subject membership probability is not dependent merely on the absolute magnitude of regional group differences in AIs. Thus, minimization of residuals in a "control" region appears as much important as detection of asymmetry differences in affected regions for accurate group discrimination.

Using cut-off 2, both before and after PVC, all controls and all patients were correctly classified to their respective membership group, i.e. accuracy was 100% in both cases. Note that these values were obtained after cross validation with the leave-one-out procedure. Even though this might be seen as a very optimistic result, we note that the current validation process was performed in a small sample size and that replications of the current findings in larger cohorts are necessary. In larger samples, more realistic results below 100% are expected. Thus, the 100% value obtained in this study should not be taken literally.

Didelot et al. previously tried to validate visual analysis of 5-HT_{1A} receptor PET images using [^{18}F]MPPF [6]. A specific pattern was encountered in the TLE patients, consisting of a BP_{ND} [25] decrease involving hippocampus, amygdala and temporal pole altogether. They found that visual analysis showed a low rate (7%) of disagreement between the three examiners. However, combining the results from the visual analysis and the surgical outcome, the authors estimated that the area of BP_{ND} decrease coincided with the epileptogenic zone only in 40% of TLE patients. This low precision was due to 47% of patients who showed BP_{ND} decreases in the ipsilateral insula, extending beyond the mesial temporal regions [6]. In our study, insula was

selected as significant predictor only after PVC by both cut-off 1 and cut-off 2. Therefore, it is possible that correction of AIs increase after PVC helps to enhance group discrimination by identifying patients in whom insular 5-HT_{1A} specific binding is initially reduced. Results of the visual analysis by Didelot et al. were similar to ours: in fact they reported no false positives and false negative rate inferior to 10% [6]. However, despite these promising features of visual analysis, in the neuroimaging field some semiquantitative analysis in support of visual analysis is common and desirable.

The first clinical applications of the discriminant analysis date back to the early ’90s and it was used in the clinical context of early detection of neurodegenerative disorders using FDG. In its first applications, discriminant analysis was coupled to multiple regression. In neurodegenerative disorders, early diagnosis may be difficult if based only on clinical examination and FDG PET may be negative or inconclusive. Thus, more sophisticated statistical analyses were felt of critical importance to the development of effective treatment strategies. In 1991, Clark et al. first applied discriminant analysis to a group of Huntington’s disease patients and healthy controls [38]. In their study, Clark et al. determined, using regression analysis, the most accurate set of cortical and thalamic estimates of the cerebral metabolic rate for glucose (CMRglu) for predicting caudate metabolism in normal subjects. Then, they applied these parameters to Huntington’s disease patients to quantify the predicted CMRglu in caudates. Finally, they calculated the residuals between predicted and observed CMRglu in caudates and used these residuals as input to the discriminant function analysis. They obtained an overall correct classification of 98.3% [38].

Azari et al. also used multiple regression coupled with discriminant analysis. They first calculated the correlation coefficients among a set of predetermined regions relevant to the pathogenesis of Alzheimer’s disease and applied these correlation coefficients to discriminant analysis. They obtained a correct classification rate of 87%. The discriminant function was

subsequently successfully applied to an initial FDG PET scan of a subject at risk for Alzheimer's disease that was considered visually unremarkable allowing a posteriori diagnosis [39].

Our approach differs from the previously adopted ones since, rather than using regression analysis, we calculated the input of the discriminant analysis from the difference of the AIs in TLE patients and two cut-offs obtained from healthy control values. Although our method is somehow different in the statistical procedures, it is conceptually similar because the input to the discriminant function is derived from a mathematical comparison between patients and control values. Our approach has the noteworthy advantage of easier implementation.

Having conceded reservations about the finding of 100% correct classification, we should underline that previously cited studies with FDG also reported very high results, ranging from 87% to 98.3% [38, 39]. The sensitivity of the discriminant function to the identification of group membership depends on the baseline differences of the two groups from which the function is derived. Derivation of the discriminant function from subtle differences in the variable of interest is ideal when early detection is desired, while derivation in presence of larger group differences is expected to increase accuracy. Differences in CMRglu between early stages of Alzheimer's disease or Huntington's disease and healthy controls may not be detectable, or may be subtle and they are likely smaller than differences in 5-HT_{1A} receptor concentration between typical, drug-resistant TLE patients and normal controls. However, identification of significant asymmetries in 5-HT_{1A} receptor concentration in selected TLE patients may also be difficult since 5-HT_{1A} receptor concentration may change as a function of some clinical and imaging variables: for example, it is less marked in patients with normal MR and with shorter duration of disease, while other patients may have nonlateralized (that is, negative) [^{18}F]FCWAY scans [40].

We used PVC to correct the bias introduced by MTS and brain atrophy. PVC is a sophisticated technique that necessitates highly qualified technical skills and it is unlikely to be available in all epilepsy centers where 5-HT_{1A} receptor ligands are being used. Cut-off 2

performed with equal accuracy in original and PVC-corrected [^{18}F]FCWAY images and its application is therefore feasible in all epilepsy centers.

Certain limitations of the study need to be acknowledged. First, our sample size was relatively small, especially in reference to the number of healthy controls (n=10). This constrain was determined by the fact that in the current investigation we performed a re-analysis of our previous study [5], and no further subjects could be recruited. Clark et al. performed discriminant analysis in 32 Huntington's disease patients and 26 controls [38], while Azari et al. recruited 19 Alzheimer's disease patients and 22 healthy controls. As we have previously stated, the small sample size likely positively biased the accuracy of our results. Leave-one-out cross validation was used to increase the reliability of our estimates. Replication of this experiment in a larger sample is desirable.

Another limitation of the study pertains to [^{18}F]FCWAY. This tracer has been rarely used across the world in last ten years owing to significant defluorination and production of the metabolite [^{18}F]FC acid that crosses the blood: brain barrier, which require logistically complex correction strategies [13, 15]. Many other 5-HT$_{1A}$ ligands are currently available and some of them have been used for TLE, including [^{11}C]WAY-100635 [3, 7] and [^{18}F]MPPF [1, 2, 6]. The generalization of our results to other tracers requires some discussion. The reliability of the discriminant function depends primarily from the identification of a disease-specific pattern. Unpaired studies performed with different tracers consistently reported that reductions of 5-HT$_{1A}$ receptor specific binding in ipsilateral mesial, and to a lesser extent lateral, temporal regions, and in the raphe [1-7, 27]. Sometimes, reduction of the binding potential has also been reported in contralateral mesial temporal regions and insula [5, 6]. Thus, these data seem to support the existence of a reproducible 5-HT$_{1A}$ receptor disease-specific pattern. Detection of single subject or group differences also depends on the variability of the tracer. The variability of [^{18}F]FCWAY in comparison to other 5-HT$_{1A}$ ligands was analyzed in detail in a previous our study [23].

Briefly, [^{18}F]FCWAY BP_F values in control subjects show regional percent coefficient of variation (%CV) ranging from 21% to 35% [5, 23]. With [^{11}C]WAY-100635 lower variability (regional %CV ranging from 14% to 23%) was reported by Rabiner et al. [41]. Parsey et al. using the same tracer, reported %CV ranging from 22% to 53% (hippocampus), whereby hippocampus was the only region with %CV greater than 35% [42]. Passchier et al. reported %CV of [^{18}F]MPPF up to 33% [43]. Finally, [^{18}F]Mefway is another 5-HT$_{1A}$ ligand that has been used in an animal model of epilepsy [44], but not in epileptic patients. Hillmer et al. using [^{18}F]Mefway reported %CV equal to 49% in both amygdala and raphe, and lower values, ranging from 8% to 38%, in the remaining structures [45]. Thus, [^{18}F]FCWAY variability does not appear to be higher than what reported for other 5-HT$_{1A}$ receptor ligands using BP_F or different binding indices. Based on these premises, it is reasonable to expect that results similar to ours could be obtained in larger samples using other 5-HT$_{1A}$ receptor ligands. Independently from the results of the discriminant analysis, cut-off 2, which is a more restrictive criterion than cut-off 1, is clinically more appropriate in the presurgical evaluation of TLE patients when, in consideration of the surgical resection, avoiding false positives is a more critical concern than missing true positives.

Finally, we did not apply a multiple comparison correcting for ROIs and/or for subjects. In general, Bonferroni correction is not considered necessary for discriminant analysis because the latter is a multivariate technique that considers all predictor variables simultaneously, effectively controlling for the inflation of Type I errors (false positives) without assuming your variables are independent. Bonferroni correction was not performed in two cited studies by Clark et al. and Azari et al. [38, 39].

In summary, we used linear discriminant analysis to perform a formal study to identify the best threshold of 5-HT$_{1A}$ receptor AI to use in single subject analysis of TLE patients. A cut-off

based on control mean value plus 2 SDs best distinguished with good accuracy TLE patients from healthy controls both before and after PVC.

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TABLE 1. Number of patients with AI greater than cut-off 1 and cut-off 2 for each region before and after PVC.

	Before PVC		After PVC	
Region	> cut-off 1	> cut-off 2	> cut-off 1	> cut-off 2
Superior Temporal	7	3	8	7
Middle Temporal	5	0	9	6
Inferior Temporal	8	3	11	7
Fusiform	16	10	16	10
Parahippocampus	20	19	18	11
Hippocampus	22	18	19	17
Amygdala	14	9	12	7
Insula	10	7	10	8
Frontal	7	6	8	8
Parietal	6	2	11	6
Occipital	5	1	5	1

TABLE 2. Subject identification (C=Control, P=Patient), probability of being classified as TLE patient using the 2 different cut-offs of AIs of [^{18}F]FCWAY images before and after PVC. We arbitrary showed the probability of being classified as TLE patient: probability scores >.5 classify subject as TLE patient, while probability scores <.5 classify subject as healthy control. * indicates wrong membership identification.

	Before PVC		After PVC	
Subject	Cut-off 1	Cut-off 2	Cut-off 1	Cut-off 2
C1	.00123	.00000	.00007	.00000
C2	.00189	.00000	.00065	.00000
C3	.02807	.00011	.00249	.00001
C4	.00065	.00000	.00024	.00000
C5	.02602	.00024	.01335	.00005
C6	.00659	.00008	.00038	.00000
C7	.18953	.00192	.04425	.00074
C8	.26805	.00428	.06451	.00115
C9	.00029	.00000	.00138	.00000
C10	.01131	.00028	.00018	.00000
P1	.99929	.99999	.99994	1.00000
P2	.99795	1.00000	.99867	1.00000
P3	.27074*	.98845	.38257*	.97690
P4	.93371	.99930	.98180	.99994
P5	.99811	1.00000	.99914	1.00000
P6	.99978	1.00000	.99876	.99999
P7	.99996	1.00000	1.00000	1.00000
P8	.99416	.99997	.96558	.99958
P9	.99371	1.00000	.99958	1.00000
P10	.99772	.99993	.99997	1.00000
P11	.66987	.99855	.87471	.99918
P12	.97996	.99996	.97620	.99984
P13	.96194	.99989	.99605	.99998
P14	.45561*	.99177	.90766	.99954
P15	.99850	.99997	.99831	.99999
P16	.99996	1.00000	1.00000	1.00000
P17	.99435	1.00000	.99941	1.00000
P18	.63711	.99950	.88720	.99959
P19	.99790	1.00000	.99999	1.00000
P20	.31184*	.99683	.84165	.99870
P21	.99984	1.00000	.99999	1.00000
P22	.98811	.99996	.99530	.99999