

## PRIORITY COMMUNICATION

## Serotonin 1B Receptor Imaging in Alcohol Dependence

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**Background:** Although animal models suggest that alcohol dependence (AD) is associated with elevations in the number of serotonin 1B receptors (5-HT<sub>1B</sub>R), 5-HT<sub>1B</sub>R levels have not been investigated in people with AD. The selective 5-HT<sub>1B</sub>R antagonist radioligand, [<sup>11</sup>C]P943, permits in vivo assessment of central 5-HT<sub>1B</sub>R binding potential (BP<sub>ND</sub>) with positron emission tomography. Because of its central role in AD, we were particularly interested in ventral striatal 5-HT<sub>1B</sub>R BP<sub>ND</sub> values.

**Methods:** Twelve medication-free, recently abstinent (at least 4 weeks) patients with AD (mean age 35.2 ± 10.2 years, 5 women) and 12 healthy control subjects (HC) (mean age 30.6 ± 9.2 years, 5 women) completed [<sup>11</sup>C]P943 positron emission tomography on a high-resolution research tomograph. Individual magnetic resonance imaging scans were collected to exclude individuals with anatomical abnormalities and for coregistration. Imaging data were analyzed with a multilinear reference tissue model.

**Results:** Ventral striatal 5-HT<sub>1B</sub>R BP<sub>ND</sub> values (2.01 ± .57% and 1.55 ± .09%, respectively; 29% between-group difference, *p* = .006) were increased in AD compared with HC subjects. No influence of demographic or clinical variables or amount of injected radiotracer was observed.

**Conclusions:** This study provides the first evidence that AD in humans is, like in rodent models, associated with increased levels of ventral striatal 5-HT<sub>1B</sub>Rs.

**Key Words:** Alcohol dependence, brain imaging, human subjects, positron emission tomography, serotonin 1 B receptor

Disturbances in the regulation of brain serotonin (5-HT) systems have been implicated in diminished inhibitory control of behavior, including pathological alcohol use (1–3). However, the specific mechanisms through which 5-HT systems are dysregulated in alcohol dependence (AD) remain unclear. For example, recent studies have yielded conflicting results regarding the regulation of 5-HT transporter (4–6) and 5-HT<sub>1A</sub> receptor function (4) in AD. Growing evidence suggests that serotonin 1B receptors (5-HT<sub>1B</sub>Rs) modify the reinforcing, intoxicating, and discriminative stimulus effects of alcohol and regulate its voluntary intake (7). The ventral striatum (VST) including globus pallidus and the nucleus accumbens area might be particularly important regions for the modulatory role of 5-HT<sub>1B</sub>Rs on alcohol-related behaviors (8). In rodents, increased expression of 5-HT<sub>1B</sub>R in the VST modulates drinking behavior (i.e., increases ethanol consumption and shifts the animal's preference for a stronger concentration of ethanol) (7). In humans, polymorphisms in the 5-HT<sub>1B</sub>R gene have been linked to antisocial AD (9), although not unequivocally (10).

The purpose of the current study was to determine whether human AD would be associated, as predicted by animal models, with increased 5-HT<sub>1B</sub>R levels in the globus pallidus and the nucleus accumbens area. The development of the selective 5-HT<sub>1B</sub>R radioligand, [<sup>11</sup>C]P943, permitted in vivo assessment of

central 5-HT<sub>1B</sub>R binding during positron emission tomography (PET) imaging.

## Methods and Materials

### Subjects

Twelve alcohol-dependent subjects (5 women, mean age 35.2 ± 10.2 years, range 22–48) meeting DSM-IV criteria for current AD and 12 healthy control subjects (5 women, mean age 30.6 ± 9.2 years, range 19–44) were recruited through public advertisement (Table 1). Individuals reporting abuse or dependence of any other substance other than alcohol or nicotine were excluded. The protocol was approved by the Yale University School of Medicine Human Investigation Committee, the Human Subjects Subcommittee of the Veterans Affairs Connecticut Healthcare System, the Magnetic Resonance Research Center, and the Yale New Haven Hospital Radiation Safety Committee. Written informed consent was obtained from all participants after full explanation of study procedures. All participants were evaluated by physical examination, electrocardiogram, standard laboratory tests, urine analysis, and toxicology. Subjects with significant medical or neurological conditions and with history of head injury with loss of consciousness were excluded from the study. The AD subjects were admitted to the Clinical Neuroscience Research Unit at Connecticut Mental Health Center for detoxification and to ensure their medication-free status for at least 4 weeks before imaging. Urine toxicology and Breathalyzer were collected repeatedly during the inpatient admission to the Clinical Neuroscience Research Unit preceding the imaging studies and on the days of the magnetic resonance (MR) imaging and PET scans.

### Scanning and Imaging Procedures

Subject preparation for the PET scan consisted of indwelling venous catheter placement. A transmission scan with a <sup>137</sup>Cs point source was obtained before the emission scan. The PET scans were acquired for 120 min at rest with a single intravenous injection of high specific activity [<sup>11</sup>C]P943, a selective 5-HT<sub>1B</sub> receptor antagonist radiotracer (11), on a high-resolution research tomograph PET scanner (207 slices, resolution <3 mm

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**Table 1.** Demographic Data and Clinical Characteristics

Group	Healthy Control (n = 12)	Alcohol Dependence (n = 12)	p
Age (yrs)	30.6 ± 9.2 range: 18–49 yrs	35.2 ± 10.2 range: 22–51 yrs	.25 —
Gender	5 F; 7 M	5 F; 7 M	1
Race	1 Mix/1 AS/10 C	1 Mix/4 AA/7 C	—
BMI	25.2 ± 4.0	25.5 ± 5.0	.87
Smoking Status	10 N; 2 S	3 N; 9 S	.01*
Age at First Drinking (yrs)	—	13.3 ± 4.3	—
Total yrs of Drinking	—	15.3 ± 9.9	—
Injected Dose (MBq)	668.4 ± 30.2	695.3 ± 51.4	.17
Specific Activity (MBq/nmol)	9.8 ± 3.4	8.7 ± 2.6	.39
Injected Mass (μg)	1.6 ± .8	1.8 ± .6	.32

Data represent mean ± SD. The *p* values by independent unpaired *t* tests, except gender and smoking status by Fisher's  $\chi^2$  tests.\*

F, female; M, male; Mix, mixed; AS, Asian-American; C, Caucasian; AA, African-American; BMI, body mass index; N, nonsmoker; S, smoker.

full-width-at-half-maximal in three-dimensional acquisition mode). Dynamic scan data were reconstructed with corrections (attenuation, normalization, scatter, randoms, and dead time). Motion correction of PET data were performed by coregistering each reconstructed frame to an early summed image (0–10 min after injection) with a 6-parameter mutual information algorithm and FMRIB's Linear Image Registration Tool (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, United Kingdom).

Magnetic resonance images were obtained for each subject on a Siemens 3-T Trio system (Siemens Medical Solutions, Malvern, Pennsylvania) to exclude individuals with anatomical abnormalities and for coregistration. A second summed image (0–10 min after injection) was created from the motion-corrected PET data and registered to the subject's MR image, which in turn was registered (12-parameter affine transformation) to an MR template (Montreal Neurological Institute space). The globus pallidus/nucleus accumbens region of interest was taken from the template (Anatomical Automatic Labeling [12] for SPM2 [<http://www.fil.ion.ucl.ac.uk/spm/software/spm2/>]) and applied to the PET data to produce time-activity curves for cerebellum. Pixel × pixel analysis was performed with the multilinear reference tissue model (13) to produce images of binding potential ( $BP_{ND}$ ) (14). The interpretation of  $BP_{ND}$  is  $f_{ND} \times B_{avail}/K_d$ , where  $f_{ND}$  is the tracer-free fraction in a region without specific binding,  $B_{avail}$  is the unoccupied receptor concentration, and  $K_d$  is dissociation equilibrium constant of the tracer. Cerebellum was used as the reference region, because it is practically devoid of 5-HT<sub>1B</sub>Rs (15). Assuming that there is no change in affinity or nonspecific binding between subject groups, changes in  $BP_{ND}$  were interpreted as changes in receptor concentration. The  $BP_{ND}$  values from the multilinear reference tissue model have provided highly comparable results to those obtained with arterial input functions (16).

### Statistical Analysis

Unpaired *t* tests were used to compare clinical and demographic variables, and a Mann–Whitney *U* test was used to compare 5HT<sub>1B</sub>  $BP_{ND}$  values in the VST between AD and control groups. All tests were performed two-tailed, with results considered significant at *p* < .05. Data are presented as means ± SD.

### Results

The 5-HT<sub>1B</sub>  $BP_{ND}$  was significantly elevated in the AD group relative to the healthy control group in the globus pallidus/

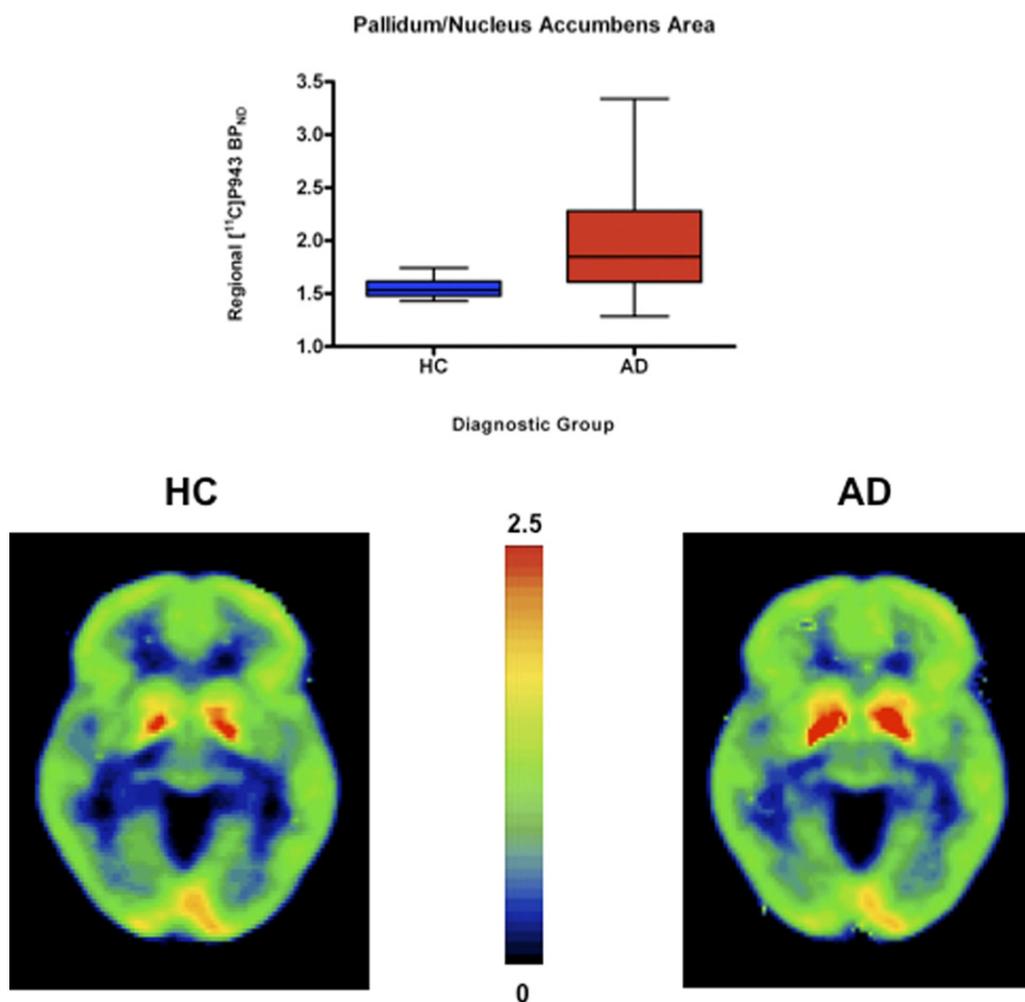
nucleus accumbens area ( $2.01 \pm .57$  and  $1.55 \pm .09$ , respectively; 29% between-group difference; *U* = 119.0, *z* = 2.71, *p* = .006) (Figure 1), with effects found bilaterally (left hemisphere: 35% between-group difference, and right hemisphere: 24% between-group difference). None of the clinical measures or injection parameters correlated with 5-HT<sub>1B</sub>  $BP_{ND}$  in either group.

### Discussion

The principal observation of this study was that ventral striatal 5-HT<sub>1B</sub>  $BP_{ND}$  was increased in AD subjects who were scanned during early abstinence relative to a group of HC subjects. This increase was not directly related to features of the history of alcohol consumption among patients in this study. Our data do not clarify whether these differences are a preexisting condition in AD patients or, alternatively, a consequence of the disorder.

The mechanism underlying the increase in ligand-binding to 5-HT<sub>1B</sub>Rs is not evident from the current data. The increases in 5-HT<sub>1B</sub>  $BP_{ND}$  might reflect an overexpression of 5-HT<sub>1B</sub>Rs or a higher affinity of these receptors. Alternatively, it is possible that low synaptic 5-HT concentrations could leave more unoccupied 5-HT<sub>1B</sub>Rs for the radioligand [<sup>11</sup>C]P943-binding, and ligands that are sensitive to endogenous 5-HT might be developed in the future. In either case, alterations in 5-HT<sub>1B</sub>R function might contribute to AD by influencing 5-HT input to the VST via their role as 5-HT terminal autoreceptors, dopaminergic input to the striatum via the role of these receptors as heteroreceptors on  $\gamma$ -aminobutyric acid terminals within the ventral tegmental area, and glutamatergic activity within the VST via heteroreceptors on corticofugal projections (17). We acknowledge that PET imaging cannot discriminate between pre- and postsynaptic receptors and auto- and heteroreceptors and only provides a measure of the total number of receptors in a region of interest. Emerging evidence, however, suggests that 5-HT<sub>1B</sub>Rs in the nucleus accumbens shell neurons specifically are involved in the drug-rewarding processes of ethanol (7) and other drugs of abuse (18).

A limitation of the present study is the difference in nicotine use between groups. Given the high prevalence of nicotine use among AD patients and to study a representative cohort of AD, we decided to accept smokers into the study. We acknowledge, however, that our sample size is currently too small to specifically address the issue of comorbidity between smoking and AD and therefore do not know whether it affects the outcome of the study.



**Figure 1.** In the upper row, the box plot showing significant differences in ventral striatum, including the globus pallidus and nucleus accumbens area [<sup>11</sup>C]P943 binding potential ( $BP_{ND}$ ) between patients with alcohol dependence (AD) and healthy control subjects (HC), is presented. In the lower row, the average [<sup>11</sup>C]P943  $BP_{ND}$  coregistered positron emission tomography images illustrate increased ventral striatum [<sup>11</sup>C]P943  $BP_{ND}$  in AD (right) relative to HC (left).

Altogether, the presented preliminary data suggest an important role for ventral striatal 5-HT<sub>1B</sub>Rs in AD. Our data do not clarify, however, whether an overexpression of 5-HT<sub>1B</sub>Rs is a biomarker for AD specifically or is associated with drug reward responses as well as increased vulnerability to substance use disorders in general (19). Future research will be needed to determine whether these alterations are pre-existing or a consequence of AD and whether 5-HT<sub>1B</sub>Rs might be productively targeted to treat AD.

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