Exaggerated 5-HT1A but Normal 5-HT2A Receptor Activity in Individuals Ill with Anorexia Nervosa

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Background: Many studies have found disturbances of serotonin (5-HT) activity in anorexia nervosa (AN). Because little is known about 5-HT receptor function in AN, positron emission tomography (PET) imaging with 5-HT receptor-specific radioligands was used to characterize 5-HT1A and 5-HT2A receptors.

Methods: Fifteen women ill with AN (ILL AN) were compared with 29 healthy control women (CW); PET and [11C]WAY100635 were used to assess binding potential (BP) of the 5-HT1A receptor, and [18F]altanserin was used to assess postsynaptic 5-HT2A receptor BP. [15O] water and PET were used to assess cerebral blood flow.

Results: The ILL AN women had a highly significant (30%–70%) increase in [11C]WAY100635 BP in prefrontal and lateral orbital frontal regions, mesial and lateral temporal lobes, parietal cortex, and dorsal raphe nuclei compared with CW. The [18F]altanserin BP was normal in ILL AN but was positively and significantly related to harm avoidance in suprapragenual cingulate, frontal, and parietal regions. Cerebral blood flow was normal in ILL AN women.

Conclusions: Increased activity of 5-HT1A receptor activity may help explain poor response to 5-HT medication in ILL AN. This study extends data suggesting that 5-HT function, and, specifically, the 5-HT2A receptor, is related to anxiety in AN.

Key Words: Anorexia nervosa, anxiety, 5-HT1A, 5-HT2A, positron emission tomography, serotonin

Anorexia nervosa (AN) is a disorder of unknown etiology that most commonly begins during adolescence in females. This illness is characterized by the relentless pursuit of thinness, obsessive fears of being fat, and aberrant eating behaviors, such as restrictive eating, and in some individuals, episodes of purging or binge eating (or both; American Psychiatric Association 1994). Anorexia nervosa has the highest death rate of any psychiatric illness, and many individuals have a chronic course (Sullivan *et al.* 1998). Despite the seriousness of the illness and the concerns of family members, individuals with AN often deny their illness and have little motivation to eat and gain weight. A lack of understanding of the pathophysiology of this disorder has hindered the development of effective treatments.

Large-scale family and twin studies suggest that heritable factors (Bulik *et al.* 1998; Klump *et al.* 2001) contribute to a susceptibility to develop AN. Several lines of evidence support the possibility that altered central nervous system (CNS) serotonin (5-HT) activity could be such a susceptibility factor. Considerable studies, using indirect methods such as hormonal response, show that disturbances of 5-HT activity are common in individuals who are ill with AN (ILL AN; Brewerton *et al.* 1990, Brewerton and Jimerson 1996; Goldbloom *et al.* 1990; Goodwin *et al.* 1989; Hadigan *et al.* 1995; Jimerson *et al.* 1997; Kaye *et al.* 1988, 2001; Levitan *et al.* 1997; Walsh *et al.* 1998; Wolfe *et al.* 1997). Disturbed 5-HT activity could contribute to altered appetite (Blundell 1984; Leibowitz *et al.* 1986), anxious and obsessional behaviors, and extremes of impulse control (Barr *et al.* 1992; Cloninger 1987; Higley *et al.* 1997; Kaye 1997; Lucki 1998; Mann 1999; Soubrie 1986).

The 5-HT1A and 5-HT2A receptors are of interest in AN because they have been implicated in the modulation of feeding, anxiety, depressed mood, and impulse control (Arango *et al.* 1995; Bonhomme *et al.* 1998; Cervo *et al.* 2000; De Vry *et al.* 2000; Mann 1999; Matsubara *et al.* 1991; Olivier *et al.* 2001; Simansky 1996; Stockmeier 1997). Previous studies using other types of brain imaging technologies in ILL AN, have identified alterations (e.g., hypoperfusion) in temporal, cingulate, frontal, and parietal regions (Ellison *et al.* 1998; Gordon *et al.* 1997, 2001). These and other brain regions are known to contain postsynaptic 5-HT2A receptors (Burnet *et al.* 1997; Saudou *et al.* 1994) and 5-HT1A receptors (Burnet *et al.* 1997; Saudou and Hen 1994).

The nature of 5-HT disturbances in AN and bulimia nervosa (BN) has been poorly understood because of the inaccessibility of the CNS in humans and the complexity of 5-HT neuronal activity. The development of new selective tracers for the 5-HT system has made in vivo studies of 5-HT receptor function possible using positron emission tomography (PET) brain imaging. This study used PET imaging with the radioligand [18F]altanserin to assess binding potential (BP) of 5-HT2A receptors and of [11C]WAY100635 to assess the 5-HT1A receptor.

We have found persistent alteration of 5-HT1A and 5-HT2A receptors in recovered AN subjects in our PET imaging studies (Bailer *et al.* 2004, 2005; Frank *et al.* 2002). Other studies (Kaye *et al.* 1991; O'Dwyer *et al.* 1996; Ward *et al.* 1998) show that a

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disturbance of other aspects of 5-HT functional activity persists in individuals with eating disorders after they are recovered, as indicated by normal weight, nutrition, and menstrual status. The purpose of this study was to characterize 5-HT1A and 5-HT2A receptor activity in the ill state, when individuals with AN were malnourished and underweight. It is possible that the ill state might exaggerate 5-HT receptor dysfunction, given the influences of food deprivation on tryptophan (Haleem and Haider 1996; Huether *et al.* 1997; Kang *et al.* 2001; Young *et al.* 1981) and hormones that affect 5-HT receptor activity (Carrasco *et al.* 2004; Moses *et al.* 2000; Moses-Kolko *et al.* 2003; Rupprecht 2003; Wihlback *et al.* 2004).

In addition, this study assessed cerebral blood flow in these underweight and malnourished subjects. Previous studies in ILL AN have found hypoperfusion in temporal, cingulate, frontal, and parietal regions (Ellison *et al.* 1998; Gordon *et al.* 1997, 2001). Regional cerebral blood flow (rCBF) could contribute to alterations of 5-HT1A and 5-HT2A measurements. We measured rCBF using [15O] water and PET.

Methods and Materials

Subjects

Fifteen women who were ILL with AN (8 restricting type AN [RAN] and 7 bulimic-type AN [BAN]) were recruited. Subjects were currently being treated in the eating disorders treatment program at the Western Psychiatric Institute and Clinic (Pittsburgh, Pennsylvania) or were recruited through advertisements. All subjects underwent four levels of screening: 1) a brief (phone) screening; 2) an intensive screening assessing psychiatric history, lifetime weight and exercise, and menstrual cycle history as well as eating pattern for the past 12 months; 3) a comprehensive assessment using structured (Structured Clinical Interview for DSM-IV Axis I Disorders [SCID-I], First *et al.* 1996) and semistructured (Structured Clinical Interview for DSM-IV Axis II Disorders [SCID-II], First *et al.* 1997) interviews; and 4) a face-to-face interview with a psychiatrist. Subjects must not have used psychoactive medication such as antidepressants or met criteria for alcohol or drug abuse or dependence within 3 months of the study.

Twenty-nine healthy control women (CW) were recruited through local advertisements. The CW had no history of an eating disorder or any psychiatric, serious medical, or neurological illness. They had no first-degree relative with an eating disorder. They had normal menstrual cycles and had been within normal weight range since menarche. The CW were not on medication, including herbal supplements. Both ILL AN and CW were included if they were taking birth control pills.

This study was conducted according to local institutional review board regulations, and all subjects gave written informed consent. The PET imaging was performed during the first 10 days of the follicular phase for all CW. The follicular phase was determined by history. Details on assessments are described elsewhere (Bailer *et al.* 2004, 2005; Wagner *et al.* 2006). Data on 16 CW for [11C]WAY100635 BP and [18F]altanserin BP have been previously reported (Bailer *et al.* 2004, 2005), but no data on these ILL AN have been reported. A total of 29 CW and 15 ILL AN were studied, but not all subjects had all three studies available. Whenever possible, the subjects were studied on a day when individuals had PET scans with each radioligand in the following order: [15O]water, [11C]WAY100635, [18F]altanserin.

Image Acquisition

Magnetic resonance (MR) imaging and PET imaging were performed as previously described for arterial-based dynamic imaging of [11C]WAY100635 binding to 5-HT1A receptors (Bailer *et al.* 2005; Meltzer *et al.* 2004) and of [18F]altanserin binding to 5-HT2A receptors (Bailer *et al.* 2004). The [11C]WAY100635 and [18F]altanserin were synthesized according to established methods (Lemaire *et al.* 1991; McCarron *et al.* 1996; Meltzer *et al.* 2001; Price *et al.* 2001a, 2001b). A slow bolus intravenous injection of 13.8 ± 2.1 mCi high-specific activity [11C]WAY100635 was administered, and dynamic three-dimensional emission scanning with arterial blood sampling (34 sample input function) was performed over 60 min in 13 ILL AN and 21 CW (a longer 90 min acquisition was collected in 7 of the 13 ILL AN and 14 of the 21 CW subjects). The 60 min acquisitions were performed in earlier studies. Later studies used 90 minute acquisition to verify stability in the BP measures in areas such as the raphe (Parsey *et al.* 2000). A metabolite corrected input function was determined, as previously described (Meltzer *et al.* 2004). A slow bolus intravenous injection of 10.3 ± .8 mCi high-specific activity [18F]altanserin and dynamic 2D emission scanning with arterial blood sampling (input function) was performed over 90 min in 12 ILL AN and 25 CW.

A subset of 13 CW and 11 ILL AN was additionally scanned with the radiotracer [15O] water. Following slow bolus intravenous injection of 50 mCi of [15O] water, a 20-frame dynamic emission scan was acquired over 3 min in two-dimensional imaging mode, as previously described (Frank *et al.* 2000).

Data Analysis

The regions of interest (ROI) were hand drawn on the coregistered MR images and applied to the dynamic PET data to generate time-activity curves as previously described (Bailer *et al.* 2004, 2005). The following ROIs were selected: prefrontal cortex (Brodmann's area [BA] 10), medial orbital frontal cortex (BA 11), lateral orbital frontal cortex (BA 47), mesial–temporal cortex (amygdala–hippocampal complex), lateral temporal cortex (BA 21), supragenual cingulate (BA 24/32, five planes superior to anterior most part of genu corporis callosi), pregenual cingulate (BA 24/32, anterior to anterior most part of genu of the corpus callosum), and subgenual cingulate (BA 25, inferior to the genu of the corpus callosum) and parietal cortex (BA 7). The cerebellum (left and right hemispheres) was used as the reference region for both [11C]WAY100635 and [18F]altanserin. To reduce noise, right and left regions were combined.

For [11C]WAY100635, the dorsal raphe nucleus was also sampled (Bailer *et al.* 2005). Based on the coregistered MRs, the brainstem was subdivided into a rostral (midbrain and upper pons) and caudal region (medulla and pons) to approximate the dorsal and median raphe nuclei, respectively. The raphe nuclei cannot be delineated on MR and these ROIs were directly identified on the PET image using circular fixed 6-mm radius ROIs (for all subjects) placed over the area of highest radioactivity (Drevets *et al.* 2000). The inferior border of the dorsal raphe nucleus was identified by the interpeduncular cistern.

An MR-based CSF correction method was applied to correct the PET data for the dilutional effect of expanded CSF spaces accompanying normal aging and disease-related cerebral atrophy (Meltzer *et al.* 1996, 1999).

[11C]WAY100635

For the arterial-based kinetic analyses, regional [11C]WAY100635 distribution volume (DV) values were determined using both the

Logan graphical method (Logan *et al.* 2001) and three-compartment model (two-tissue compartments; Parsey *et al.* 2000) that included a vascular volume term. A modified Logan analysis that applied generalized linear least squares smoothing to the regional data before analysis (Price *et al.* 2002a) was used because it effectively reduced noise-induced bias in the Logan DV as previously described for other PET radiotracers (Logan *et al.* 2001). The Logan analysis was performed on PET data acquired after 25 min with 7 or 10 data points used for the analyses of the 60- and 90-min data sets, respectively. Although the concentration of cerebellar 5-HT1A receptors is low, its influence on ROI-specific binding could not be excluded. Analyses of the cerebellar data indicated greater cerebellar DV values for ILL AN subjects relative to CW (Results). The specific 5-HT1A receptor binding measure used in this work was one that was not strongly influenced by tissue nonspecific binding. The BP measure was determined as: BP = DVROI – DVCER. This BP is dependent on plasma protein binding (f1) rather than tissue free fraction (f2) (Parsey *et al.* 2000). As a result, plasma protein binding was measured in all subjects to determine the extent to which a group difference in [11C]WAY100635 BP could be influenced by this factor.

[18F]altanserin

For the kinetic analyses, the Logan graphical method and a four-compartment model were applied using an arterial based input function on the sampled ROI data. The four-compartment modeling of the regional data used the nonspecific kinetic parameters determined from a three-compartment fit to the cerebellar data (Price *et al.* 2001b). The Logan analysis was performed for the 12- to 90-min time interval (10 data points). The regression slope value ([18F]altanserin distribution volume, DV) for each ROI was calculated (Logan *et al.* 1990). Specific 5-HT2A receptor binding was assessed using the BP measure. The BP measure was determined as: BP = (DVROI/DVCER) – 1 (Lammertsma 2002). This BP is dependent on tissue nonspecific binding (f2) rather than nonspecific effects of plasma (f1). Although the concentration of cerebellar 5-HT2A receptors is low, an influence on ROI-specific binding could not be excluded. We, therefore, also compared the cerebellar DV between groups.

[15O]water

The [15O]water data were analyzed using a one-tissue two-compartmental model (Price *et al.* 2002b) in which blood flow was measured as the clearance of [15O]water from blood to brain (K1, mL min-1 mL-1) while accounting for arterial input function timing delays. Cerebral blood flow was assessed on a regional basis (rCBF) via regional values of K1.

Statistical Analysis

Standard statistical software packages (SAS ver. 8.2) were used for all analyses. Pearson correlation coefficients were also computed and exact significance levels based on Monte Carlo methods are reported. All values are expressed as mean ± SD. As a level of significance, a p value of $p < .05$ was selected. We adjusted for multiple comparisons using the method of false discovery rate (Benjamini and Hochberg 1995).

Comparisons between CW and ILL AN for [18F]altanserin Logan graphical method and compartmental modeling as well as for rCBF were made using one-way analyses of variance (ANOVAs). To explore the effect of age on the 5-HT2A receptor binding results, we also tested for group differences while adjusting for age. These analyses were completed using a linear model with the BP value as the outcome and group membership and age as predictors. The comparison for [11C]WAY100635 BP Logan graphical analysis and compartmental modeling was analyzed using nonparametric statistics (Wilcoxon Rank–Sum test) because there was not homogeneity of variance between the two groups.

A repeated-measures ANOVA using a contrast transformation was applied to explore potential group differences in radiolabeled metabolites of [11C]WAY100635 and [18F]altanserin. Because sphericity tests failed, p values for the within-subject effects (time and the interaction of time × group) were adjusted using Huynh and Fedlt's estimator. For the [11C]WAY100635 metabolites, only 7 of the 13 ILL AN and 14 of the 21 CW subjects had 90-min data available, therefore, the model was run without the 90-min measurement.

Results

Subjects

The ILL AN women had the onset of their eating disorder at the age of 16.3 ± 2.6. The ILL AN and CW were of similar ages at the time of study (Table 1). Otherwise, ILL AN women and CW had highly significant differences for current body mass index (BMI) and all measures of the core symptoms of eating disorders, depression, and anxiety. The ILL AN and CW had similar cortisol and b-hydroxybutyrate (BHBA) values, but the ILL AN had significantly lower estradiol levels. Four ILL AN and 14 CW were taking birth control pills at the time of study. Twelve subjects in the ILL AN group had a history of major depressive disorder and 8 subjects had a history of obsessive–compulsive disorder. Three subjects fulfilled criteria for social phobia, two had a lifetime diagnosis of panic disorder, and four fulfilled criteria of generalized anxiety disorder. Additional lifetime comorbidity included alcohol dependence (two subjects), posttraumatic stress disorder (one subject), specific phobia (one subject), cocaine dependence (one subject), and cannabis dependence (one subject).

Plasma Data

The repeated-measures analysis of the unmetabolized fraction of [11C]WAY100635 (7 points) showed that unmetabolized [11C]WAY100635 in plasma was significantly higher in ILL AN relative to CW at time points 2 min (.68 ± .12 vs. .53 ± .13; $p = .002$), 5 min (.20 ± .05 vs. .14 ± .03; $p < .001$), and 30 min (.06 ± .01 vs. .05 ± .02; $p = .04$), but similar at time points 1, 10, 45, and 60 min. Both the group ($p = .004$) and the group × time ($p = .0006$) interaction were significant in this model. When the 90-min data of the subset of 7 ILL AN and 14 CW were included in the model, the results remained similar (data not shown).

The fraction of unmetabolized [18F]altanserin (5 points) in plasma was significantly higher in ILL AN relative to CW at time points 30 min (.64 ± .06 vs. .59 ± .08; $p = .05$), 60 min (.55 ± .07 vs. .47 ± .08; $p = .003$), and 90 min (.49 ± .07 vs. .42 ± .10; $p = .02$) minutes, but similar at time points 2 and 10 min. Both the group ($p = .04$) and the group × time ($p = .002$) interaction were significant in this model.

No significant differences in plasma protein binding (f1) of [11C]WAY 100635 were found between ILL AN ($n = 12$; f1 = .096 ± .033) and CW ($n = 17$; f1 = .087 ± .035; $p = .62$) in which these data were available.

ROI-Based Analysis

[11C]WAY100635 Studies. Thirteen ILL AN and 21 CW had PET studies with [11C]WAY100635. For both Logan graphical analysis and compartmental modeling, ILL RAN and ILL BAN had similar [11C]WAY100635 BP values for ROIs, so the groups were

Table 1. Demographic Data and Assessments

	CW ($n = 29$)		ILL AN ($n = 15$)		Asymptotic Significance[a]
	Mean	SD	Mean	SD	
Age (years)	25.67	5.85	24.84	4.89	.776
Current BMI (kg/m^2)	22.09	1.68	15.91	.92	<.001
AN Onset (years of age)	NA	NA	16.29 (14)	2.64	
Estradiol (pg/mL)	44.44 (27)	51.15	19.87	38.30	.001
β-hydroxybutyrate (BHBA) (mmol/L)	.07 (25)	.04	.10	.10	.736
Cortisol (mcg/dL)	16.30 (27)	6.10	18.53	4.94	.273
Depression (BDI; Beck *et al.* 1961)	1.30 (27)	1.51	20.64 (14)	11.72	<.001
Trait Anxiety (STAI; Spielberger *et al.* 1970)	27.97	6.73	54.07 (14)	16.90	<.001
Novelty Seeking (TCI; Cloninger 1987)	20.90	5.07	16.00 (13)	7.59	.037
Harm Avoidance (TCI)	10.69	4.39	21.75 (12)	5.33	<.001
EDI 2—Drive for Thinness ("worst ever"; Garner 1990)	.66	1.32	15.64 (14)	5.65	<.001
Yale–Brown Obsessive–Compulsive Scale (YBOCS; Goodman *et al.* 1989a, 1989b)	.54 (28)	1.527	17.36 (14)	13.6	<.001
Yale–Brown–Cornell Eating Disorders Scale (YBC-EDS) (Mazure *et al.* 1994; Sunday *et al.* 1995)	.21 (28)	.69	21.36 (14)	8.705	<.001
Perfectionism (MPS; Frost *et al.* 1990)	54.69	12.85	105.15 (13)	22.96	<.001

AN, anorexia nervosa; CW, healthy control women; BDI, Beck Depression Inventory; BMI, body mass index; EDI–2, Eating Disorder Inventory–2; ILL AN, women ill with anorexia; MPS, Multidimensional Perfectionism Scale; NA, not applicable; STAI, State-Trait Anxiety Inventory; TCI, Temperament and Character Inventory. The numbers in parentheses indicate the number of subjects with assessment. Data are missing because of noncompliance with the self-assessment or blood analysis failure.

[a]Group comparison by Wilcoxon Rank–Sum test.

Table 2. Regional [^{11}C]WAY100635 Binding Potential (BP) Between Groups

A. Logan Graphical Analysis

	CW ($n = 21$)		ILL AN ($n = 13$)				
	Mean	SD	Mean	SD	% diff.	Exact. Sig.[a]	FDR *p* Value[b]
Supragenual Cingulate	3.738	.918	4.973	2.119	+33	.082	.082
Subgenual Cingulate	4.700	1.221	7.003	2.697	+49	**.003**	**.008**
Pregenual Cingulate	4.543	1.186	6.523	2.422	+44	**.013**	**.014**
Dorsal Raphe	2.142	.556	3.402	1.142	+59	**.001**	**.005**
Lateral Orbital Frontal	3.827	.834	5.532	2.207	+45	**.011**	**.014**
Lateral Temporal	5.231	1.273	7.652	2.824	+46	**.007**	**.011**
Mesial Temporal	7.049	1.893	12.031	4.845	+71	**.001**	**.005**
Medial Orbital Frontal	4.667	1.245	7.147	2.923	+53	**.007**	**.011**
Parietal	3.912	.951	6.020	2.142	+54	**.002**	**.007**
Prefrontal	4.158	.993	6.502	2.828	+56	**.008**	**.011**

B. Compartmental Modeling

	CW ($n = 21$)		ILL AN ($n = 13$)			
	Mean	SD	Mean	SD	% diff.	Exact. Sig.[a]
Supragenual Cingulate	3.601	.774	4.870	2.174	+35	.070
Subgenual Cingulate	4.662	1.261	7.005	2.786	+50	**.005**
Pregenual Cingulate	4.463	1.229	6.533	2.569	+46	**.010**
Dorsal Raphe	2.121	.671	3.483	1.346	+64	**.002**
Lateral Orbital Frontal	3.698	.786	5.430	2.200	+47	**.008**
Lateral Temporal	5.161	1.253	7.595	2.888	+47	**.008**
Mesial Temporal	6.957	2.019	11.059	3.939	+59	**.002**
Medial Orbital Frontal	4.549	1.294	7.085	2.944	+56	**.006**
Parietal	3.854	.923	5.941	2.187	+54	**.004**
Prefrontal	4.106	.952	6.397	2.873	+56	**.007**

CW, control women; diff., difference; ILL AN, women ill with anorexia; Sig., significance.

Figures in boldface indicate significance.

[a]Group comparisons by Wilcoxon rank sum tests.

[b]Because there were a number of significant findings, *p* values were adjusted using the method of False Discovery Rate (FDR; Benjamini *et al.* 1995).

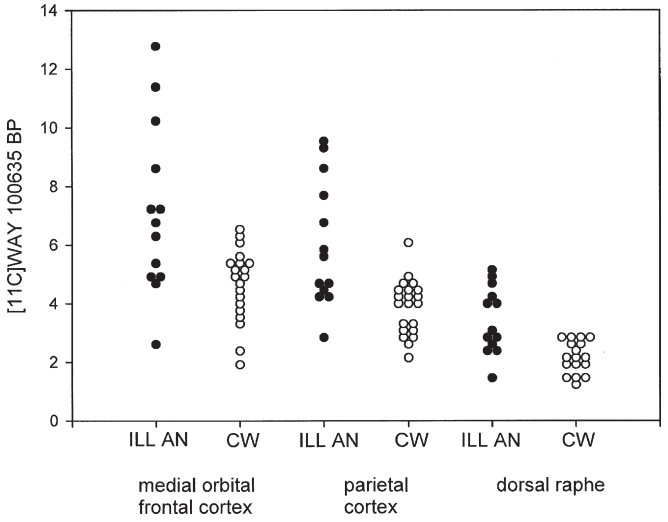

Figure 1. Scatter histograms of the [11C]WAY100635 binding potential (BP) values for control women (CW) and women ill with anorexia (ILL AN) in the medial orbital frontal cortex (left plot), parietal cortex (middle plot), and dorsal raphe (right plot).

combined (ILL AN). Compared with CW, the ILL AN had increased [11C]WAY100635 BP for all ROIs (Table 2A and 2B). Differences remained significant after correction for multiple comparisons by false discovery rate (Table 2A). Illustrative scatterplots (Figure 1) are shown for the medial orbital frontal cortex, parietal cortex, and dorsal raphe. The ILL AN had significantly higher cerebellar DV compared with CW in the Logan graphical analysis (.72 ± .13 vs. 1.0 ± .4; $p = .02$), but higher values showed only at a trend when using the compartmental modeling (.53 ± .11 vs. .73 ± .4; $p = .09$).

The temporal stability of the outcome measures was examined in the subset of subjects (14 CW and 7 ILL AN) for which a full 90 min emission data set was available. High correlations were observed between the cerebellar DV and regional BP measures calculated using the 60-min and 90-min data sets (CW: $r = .95–.99$; ILL AN: .95–.99). The bias across ROIs between the two measures ranged from 5% to 14% in CW and from 4% to 21 % in ILL AN subjects.

[18F]altanserin Studies. A total of 12 ILL AN and 25 CW had PET studies with [18F]altanserin. For both Logan graphical analysis and compartmental modeling, the ILL RAN and ILL BAN had similar [18F]altanserin BP for all ROIs, so the groups were combined. The CW and ILL AN had similar [18F]altanserin BP in all ROIs (Table 3A and B). Illustrative scatterplots (Figure 2) are shown for the medial orbital frontal and parietal cortex. The ILL AN had significantly lower cerebellar DV in Logan graphical analysis (1.41 ± .22 vs. 1.17 ± .21; $p = .005$) and compartmental modeling (1.43 + .57 vs. 1.72 + .34; $p = .005$).

[15O]Water Studies. In the subset of 11 ILL AN and 13 CW cerebral blood flow, as measured by K1, was similar in all ROIs (Table 4), including the cerebellum (CW: .51 ± .07 vs. ILL AN: .48 ± .10; $p = .42$).

Other Relationships

The ILL AN showed a positive relationship between harm avoidance and [18F]altanserin BP in the lateral orbital frontal

Table 3. Regional [18F]altanserin Binding Potential (BP) Between Groups

A. Logan Graphical Analysis

	CW ($n = 25$)		ILL AN ($n = 12$)				
	Mean	SD	Mean	SD	% diff.	Without Age Correction Sig.[a]	With Age Correction Sig.[b]
Supragenual Cingulate	1.317	.253	1.208	.331	−8	.275	.127
Subgenual Cingulate	1.584	.299	1.568	.274	−1	.883	.735
Pregenual Cingulate	1.566	.265	1.564	.256	0	.984	.704
Lateral Orbital Frontal	1.594	.323	1.466	.297	−8	.254	.097
Lateral Temporal	1.815	.288	1.749	.265	−4	.504	.191
Mesial Temporal	.581	.196	.568	.129	−2	.833	.703
Medial Orbital Frontal	1.662	.284	1.604	.293	−4	.566	.263
Parietal	1.736	.288	1.665	.303	−4	.496	.283
Prefrontal	1.745	.337	1.760	.294	1	.896	.956

B. Compartmental Modeling

	CW ($n = 25$)		ILL AN ($n = 12$)			
	Mean	SD	Mean	SD	% diff.	Without Age Correction Sig.[a]
Supragenual Cingulate	2.342	.482	2.222	.544	−5	.501
Subgenual Cingulate	2.808	.473	2.848	.599	1	.834
Pregenual Cingulate	2.735	.401	2.771	.459	1	.812
Lateral Orbital Frontal	2.569	.599	2.448	.316	−5	.536
Lateral Temporal	3.033	.532	2.917	.465	−4	.524
Mesial Temporal	.991	.373	.938	.210	−5	.659
Medial Orbital Frontal	2.858	.502	2.776	.441	−3	.642
Parietal	2.849	.483	2.785	.449	−2	.703
Prefrontal	2.773	.630	2.764	.418		.961

CW, control women; diff., difference; ILL AN, women ill with anorexia; Sig., significance.
[a]Group comparisons by one-way analysis of variance.
[b]Linear model with the binding potential value as the outcome and group membership and age as predictors.

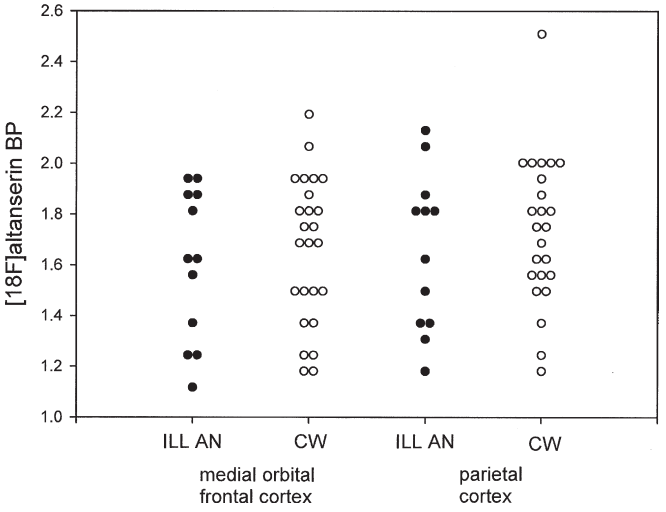

Figure 2. Scatter histograms of the [^{18}F]altanserin binding potential (BP) values for control women (CW) and women ill with anorexia (ILL AN) in the medial orbital frontal cortex (left plot) and parietal cortex (right plot).

cortex (r = .82; p = .004) and medial orbital frontal cortex (r = .88; p = .0007) (Figure 3) as well as in the supragenual cingulate (r = .68; p = .03), and parietal cortex (r = .75; p = .01). Highly significant negative correlations with age and [18F]altanserin BP were seen in both ILL AN and CW (data not shown). No relationships were found for either group between [18F]altanserin BP or [11C]WAY100635 and current BMI, plasma BHBA, estradiol, or any of the variables in Table 1. There were also no differences in [11C]WAY100635 BP or [18F]altanserin BP across ROIs between subjects who were or were not on birth control pills within each group. There was no relationship between [11C]WAY100635 BP and [18F]altanserin BP for any ROI.

Discussion

The ILL AN had a 30% to 70% increase in [11C]WAY100635 BP in brain regions where presynaptic and postsynaptic 5-HT1A receptors are known to occur. In contrast, the ILL AN had normal [18F]altanserin BP values. The [18F]altanserin BP was, however, positively related to harm avoidance in the suprapragenual cingulate, frontal, and parietal regions in ILL AN.

To our knowledge, imaging has not been previously used to assess 5-HT1A receptor activity in ILL AN. In terms of postsynaptic 5-HT2A receptor activity, Audenaert *et al.* (2003) used single photon emission computed tomography (SPECT) and 123I-5-I-R91159 and found that ILL AN had reduced binding in the left frontal, bilateral parietal, and occipital cortex. That study did not account for possible brain volume loss in ILL AN, however, so that the reduced binding may be the result of partial volume averaging, leading to an underestimation of binding per unit brain volume in the ILL AN group.

Studies from our group have assessed both of these receptors (with [11C]WAY100635 and [18F]altanserin) in individuals who had recovered from AN. In terms of 5-HT1A receptors, women recovered from bulimic-type AN (REC BAN) had a significant 20% to 40% increase in [11C]WAY100635 BP in cingulate, temporal, frontal, and parietal regions and in dorsal raphe compared with CW (Bailer *et al.* 2005). In contrast, women recovered from restricting-type AN (REC RAN) had nonsignificantly increased [11C]WAY100635 BP compared with control subjects (Bailer *et al.* 2005). In terms of 5-HT2A receptor activity, REC RAN (Frank *et al.* 2002) had reduced [18F]altanserin BP in mesial temporal and parietal cortical areas as well as in subgenual and pregenual cingulate cortex. Similarly, REC BAN (Bailer *et al.* 2004) women had reduced [18F]altanserin BP relative to control subjects in left subgenual cingulate, left parietal, and right occipital cortex.

One important question is whether there are significant differences in these 5-HT receptors between the ill state in comparison to the recovered state. Because of the possibility that RAN and BAN individuals may have differences in receptor activity, such a comparison should be carried out comparing the subtypes. First, we should note that we did not find a difference between ILL BAN and ILL RAN for either [18F]altanserin BP or [11C]WAY100635 BP values. This may be a consequence of the small sample size for both subgroups. It is also important to note that it is common for subtypes of ILL AN subjects to cross over from one diagnostic group to another over the course of the illness (Herzog *et al.* 1996). Thus, it is possible that some ILL RAN may convert to ILL BAN in the future. Still, in an exploratory analysis (data not shown), we compared these ILL subjects to the recovered subgroups (e.g., by RAN and BAN subgroups) that were previously reported (Bailer *et al.* 2004, 2005). There was no significant difference between ILL and recovered subjects for mean [11C]WAY100635 BP or mean [18F]altanserin BP values. It is important to note, however, that the analysis was underpowered (the range of power was 10% to 55%). In conclusion, modest power of these analyses and potential crossover confounds determination of whether there are significant differences in 5-HT1A or 5-HT2A receptor activity between the ill and recovered state.

Despite the abundance of data implicating 5-HT dysregulation in AN, it remains uncertain whether individuals with AN respond to medications that act on 5-HT (Kaye and Walsh 2002). That is because only a few controlled trials of medication have been done in AN. Still, there is some evidence that selective serotonin reuptake inhibitor (SSRI) medication is not useful in terms of accelerating weight gain, improving mood or obsessionality, or reducing core eating disorder symptoms (e.g., drive for thinness, etc.) in the ill state (Attia *et al.* 1998; Barbarich *et al.* 2004; Ferguson *et al.* 1999; Strober *et al.* 1999). Response to SSRI medication is, in part, associated with a desensitization of the 5-HT1A autoreceptor (Blier and de Montigny 1999). As a group, the ILL AN showed a 59% increase in [11C]WAY100635 BP in the dorsal raphe region, perhaps the highest values found for any disorder. Increased 5-HT1A raphe autoreceptors would result in reduced 5-HT neuronal firing, and thus decreased extracellular

Table 4. Regional Cerebral Blood Flow (measured by K_1)

	CW (n = 13)		ILL AN (n = 11)		
	Mean	SD	Mean	SD	Sig.[a]
Supragenual Cingulate	.53	.08	.53	.11	.96
Subgenual Cingulate	.51	.08	.49	.11	.58
Pregenual Cingulate	.56	.09	.52	.12	.39
Lateral Orbital Frontal	.62	.10	.54	.12	.09
Lateral Temporal	.48	.06	.46	.08	.44
Mesial Temporal	.45	.06	.44	.08	.68
Orbital Frontal	.56	.08	.51	.12	.24
Parietal	.55	.07	.50	.11	.19
Prefrontal	.58	.10	.53	.11	.26
Dorsal Raphe	.46	.07	.43	.12	.68

CW, control women; ILL AN, women ill with anorexia; Sig., significance.
[a]Group comparisons by one-way analysis of variance.

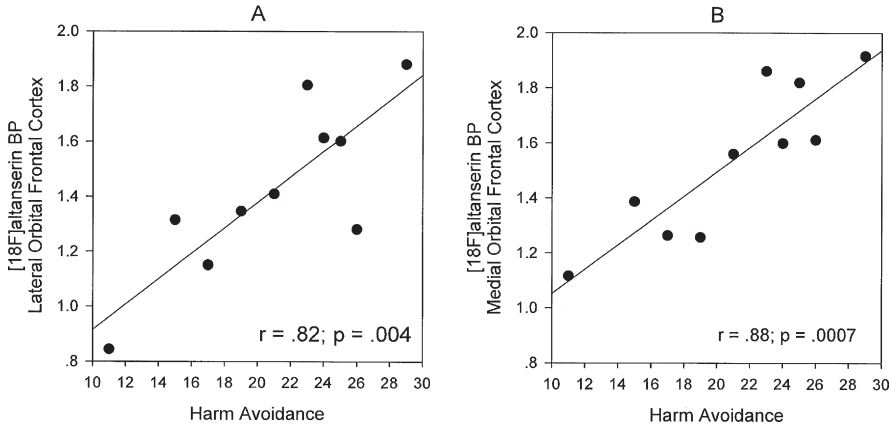

Figure 3. Correlation of harm avoidance and [^{18}F]altanserin binding potential (BP) in the **(A)** lateral orbital frontal cortex and **(B)** medial orbital frontal cortex in women ill with anorexia. *r* indicates Pearson correlation coefficient.

5-HT. This is consistent with findings that ILL AN have approximately a 30% reduction of CSF concentrations of 5-HIAA, the major 5-HT metabolite in brain (Kaye *et al.* 1988). We hypothesized that SSRIs are not effective in the ill state because SSRIs would result in little accumulation of 5-HT in the synapse and thus are not able to downregulate exaggerated 5-HT1A receptor activity.

We previously reported that REC BAN subjects had a positive relationship between [18F]altanserin BP and harm avoidance in the left subgenual cingulate, left lateral temporal cortex and mesial temporal cortex (Bailer *et al.* 2004). In the current study, we were able to replicate those findings of positive relationship of 5-HT2A activity and harm avoidance in another independent sample of subjects with eating disorders, consistent with the literature that implicates that 5-HT activity is related to measures of affective instability and impulsivity in ILL BN (Steiger *et al.* 2001a, 2001b, 2001c). The regions involved in the current study (supragenual cingulate, lateral orbital frontal cortex, medial orbital frontal cortex, and parietal cortex) were different, however. A 5-HT2A receptor binding and harm avoidance were shown to be negatively correlated in the frontal cortex in healthy subjects (Moresco *et al.* 2002) and in the prefrontal cortex in patients that attempted suicide (van Heeringen *et al.* 2003).

We did not find any difference in cerebral blood flow between the two groups. Most studies that assessed blood flow in ILL AN have used SPECT; SPECT studies in ILL AN have found both hypoperfusion in frontal, temporal, parietal, and cingulate regions (Chowdhury *et al.* 2003; Gordon *et al.* 1997; Kuruoglu *et al.* 1998; Rastam *et al.* 2001; Takano *et al.* 2001), as well as hyperperfusion in the thalamus and amygdalohippocampal complex (Takano *et al.* 2001). Again, to our knowledge none of those studies have been corrected for a possible brain volume loss in the ill state, resulting in partial volume averaging. The measured activity per unit brain volume may have been underestimated for those patient groups. Therefore, all our PET data, including those of cerebral blood flow, have been atrophy corrected, as previously described (Bailer *et al.* 2004, 2005; Meltzer *et al.* 1996, 1999). In fact, our study found significant hypoperfusion in cingulate, frontal, temporal, and parietal regions in ILL AN when blood flow data were not atrophy corrected (data not shown). Furthermore the MR-based atrophy correction factors were significantly different between CW and REC ILL AN in these cortical regions (data not shown), indicating that ILL AN subjects had a significant reduction in brain volume.

There were significant negative correlations between [18F]altanserin BP and age in both groups, but this correlation did not affect our results, which remained the same after age correction (Table 3A).

Finally, several lines of evidence show that 5-HT1A and 5-HT2A receptors interact in the brain to modulate function. In rats, 5-HT1A and 5-HT2A receptors interact robustly to regulate the inhibition of exploration of novel environments produced by either 5-HT1A and 5-HT2A receptor agonists (Krebs-Thomson and Geyer 1998). 5-HT2A and 5-HT1A receptors are highly co-localized in rodent frontal cortex (Amargos-Bosch *et al.* 2004). Postsynaptic 5-HT1A and 5-HT2A receptors mediate, respectively, the direct hyperpolarizing and depolarizing actions of 5-HT on prefrontal neurons (Santana *et al.* 2004), which in turn project to numerous cortical and subcortical areas. Thus, a balance between postsynaptic 5-HT1A and 5-HT2A receptor activity on neurons may modulate the descending excitatory input into limbic and motor structures. These data raise the speculation that postsynaptic 5-HT1A and 5-HT2A receptors fine-tune cortical systems that modulate behavioral inhibition and self-control. Mixed 5-HT2A/1A agonists (e.g., psilocybin) seem to disrupt the 5-HT1A/2A balance (Vollenweider *et al.* 1999) by driving 5-HT2A activity, thus resulting in excessive neuronal output that contributes to extremes of disinhibition, disorganization, and loss of self-control. The ILL AN may have a relative increase in 5-HT1A receptor activity compared with 5-HT2A receptor binding. Although speculative, this possible imbalance could contribute to behavioral inhibition and overcontrol commonly seen in ILL AN.

There are limitations related to the analyses of the [11C]WAY100635 and [18F]altanserin data that relate to the potential influence of nonspecific effects. The fraction of unmetabolized parent radiotracer in plasma was sometimes different for patients relative to the control subjects (only at early times for the [11C]WAY100635 data and at late times for the [18F]altanserin data). Additionally, group differences in the cerebellar DV were also evident as the average [11C]WAY100635 cerebellar DV was greater for ILL AN subjects and the average [18F]altanserin cerebellar DV was less for ILL AN subjects, relative to control subjects. The subtraction [11C]WAY100635 BP reduced the influence of tissue nonspecific binding as previously reported (Bailer *et al.* 2005; Meltzer *et al.* 2004). Group differences in the [18F]altanserin BP measure were not evident in this study. The [18F]altanserin BP was computed as a ratio (rather than by subtraction) because this parameter has been carefully evaluated and used previously in other [18F]altanserin studies (Price *et al.* 2001b). We do not understand the nature of these nonspecific

differences, but widespread physiological disturbances are well known to occur in ILL AN. Thus, attention was given in this work to assess nonspecific binding and, if possible, minimize its influence on the data analysis.

In summary, we were able to demonstrate that elevated 5-HT1A activity consistently occurs in AN subjects, whether in the ill or recovered state. Furthermore, 5-HT2A activity was found to be related to harm avoidance, although the mechanism for this relationship remains speculative.

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