ORIGINAL ARTICLE

Altered Brain Serotonin 5-HT$_{1A}$ Receptor Binding After Recovery From Anorexia Nervosa Measured by Positron Emission Tomography and [Carbonyl^{11}C]WAY-100635

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Context: Previous studies have shown that women with anorexia nervosa (AN), when ill and after recovery, have alterations of serotonin (5-HT) neuronal activity and core eating disorder symptoms, such as anxiety.

Objective: To further characterize the 5-HT system in AN, we investigated 5-HT$_{1A}$ receptor activity using positron emission tomography imaging because this receptor is implicated in anxiety and feeding behavior.

Design, Setting, and Participants: To avoid the confounding effects of malnutrition, we studied 13 women who had recovered from restricting-type AN (mean age, 23.3±5.2 years) and 12 women who had recovered from bulimia-type AN (mean age, 28.6±7.3 years) (>1 year normal weight, regular menstrual cycles, no bingeing or purging). These subjects were compared with 18 healthy control women (mean age, 25.1±5.8 years).

Intervention: The 5-HT$_{1A}$ receptor binding was measured using positron emission tomography imaging and a specific 5-HT$_{1A}$ receptor antagonist, [carbonyl-^{11}C]WAY-100635.

Main Outcome Measure: Specific 5-HT$_{1A}$ receptor binding was assessed using the binding potential measure. Binding potential values were derived using both the Logan graphical method and compartmental modeling. The binding potential in a region of interest was calculated with the formula: binding potential = distribution volume of the region of interest minus distribution volume of the cerebellum.

Results: Women recovered from bulimia-type AN had significantly ($P<.05$) increased [^{11}C]WAY-100635 binding potential in cingulate, lateral and mesial temporal, lateral and medial orbital frontal, parietal, and prefrontal cortical regions and in the dorsal raphe compared with control women. No differences were found for women recovered from restricting-type AN relative to controls. For women recovered from restricting-type AN, the 5-HT$_{1A}$ postsynaptic receptor binding in mesial temporal and subgenual cingulate regions was positively correlated with harm avoidance.

Conclusions: We observed increased 5-HT$_{1A}$ receptor binding in women who had recovered from bulimia-type AN but not restricting-type AN. However, 5-HT$_{1A}$ receptor binding was associated with a measure of anxiety in women recovered from restricting-type AN. These data add to a growing body of evidence showing that altered serotonergic function and anxiety symptoms persist after recovery from AN. These psychobiological alterations may be trait related and may contribute to the pathogenesis of AN.

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ANOREXIA NERVOSA (AN) IS A disorder of unknown etiology, which most commonly has its onset during adolescence in girls. This disorder is characterized by the relentless pursuit of thinness and obsessive fears of being fat. Two subtypes have been described: a group that restricts their eating (restricting-type AN [RAN]) and a group that alternates restrictive eating with bulimic symptoms such as episodes of purging and/or binge eating (bulimia [binging-purging]-type AN [BAN]).[1] Anorexia nervosa subtypes are thought to share some common etiological factor because they are cross-transmitted in families and have many symptoms in common.[2-4]

Several lines of evidence support the possibility that altered central nervous system serotonin (5-HT) activity contributes to the appetitive alterations found in AN.[5,6] Moreover, disturbed 5-HT activity may play a role in anxious, obsessional behaviors and extremes of impulse control.[7-12] Physiologic and pharmacologic studies show disturbances of 5-HT activity in people who are underweight with AN.[13-17]

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The nature of 5-HT disturbances in AN has been poorly understood because of the inaccessibility of the central nervous system in humans and the complexity of 5-HT neuronal activity. However, the development of selective tracers for the 5-HT system has made in vivo study of 5-HT function possible with positron emission tomography (PET). The purpose of this study was to use PET imaging with the radioligand [carbonyl-^{11}C]WAY-100635 to assess presynaptic and postsynaptic 5-HT$_{1A}$ receptor function. Studies in animals and humans raise the possibility that alterations of the 5-HT$_{1A}$ receptor may play a role in anxiety,[18-20] mood and impulse control,[12,21,22] and feeding behavior,[23-25] as well as selective serotonin reuptake inhibitor response.[26,27] Previous studies, using other brain imaging technologies, have identified potential alterations in temporal, cingulate, and frontal regions in AN.[28-30] These regions are known to contain 5-HT$_{1A}$ postsynaptic receptors.[31,32] These previous imaging studies guided our choice of brain regions to investigate in our subjects.

This study investigated women who had recovered for 1 or more years from AN. Studies of women who have recovered from an eating disorder avoid the confounding effects of malnutrition on 5-HT activity. Some, but not all, studies showed that a disturbance of 5-HT activity persists after recovery from an eating disorder.[33-35] Finally, certain behaviors, such as anxiety, perfectionism, and obsessionality, have been found to occur premorbidly and persist after recovery from AN.[36-39] Together these studies raise the possibility that altered 5-HT activity and these behavioral symptoms may be traits that contribute to a vulnerability to develop AN and are not due to malnutrition. The goal of this study was to determine whether alterations of the 5-HT$_{1A}$ receptor persisted after recovery or were related to behavioral symptoms.

METHODS

SUBJECT SELECTION

Twelve women who had recovered from BAN (REC BAN) and 13 women who had recovered from RAN (REC RAN) were recruited. Eighteen healthy control women (CW) were recruited through local advertisements. Details on sample selection and assessment are described elsewhere.[40,41]

This study was conducted according to the institutional review board regulations of the University of Pittsburgh, Pittsburgh, Pa, and all subjects gave written informed consent.

IMAGE ACQUISITION

Magnetic resonance (MR) imaging and PET imaging were performed as previously described for arterial-based dynamic imaging of [^{11}C]WAY-100635 binding to 5-HT$_{1A}$ receptors[42] during the first 10 days of the follicular phase of the menstrual cycle for all subjects. [^{11}C]WAY-100635 was synthesized according to established methods.[43,44] A bolus intravenous injection of 13.5±2 mCi (499.5±74 MBq) high specific–activity [^{11}C]WAY-100635 was administered and dynamic emission scanning with arterial blood sampling (34-sample input function) was performed across 60 minutes (a longer 90-minute acquisition was collected in 10 of 25 REC RAN and REC BAN and 11 of 18 CW). A metabolite-corrected input function was determined, as previously described.[42]

DATA ANALYSIS

The regions of interest (ROIs) were hand drawn on the coregistered MR images and applied to the dynamic PET data to generate time-activity curves. In addition to previously used ROIs,[40,41] including the cerebellum (left and right hemispheres) as a reference region, the dorsal raphe nucleus was chosen. Based on the coregistered MR images, the brainstem was subdivided into a rostral (midbrain/upper pons) and caudal region (medulla/pons) to approximate the dorsal and median raphe nuclei, respectively. The raphe nuclei cannot be delineated on MR imaging, and these ROIs were directly identified on the PET image[45] using circular, fixed, 6-mm-radius ROIs (for all subjects) placed over the area of highest radioactivity. The inferior border of the dorsal raphe nucleus was identified by the interpeduncular cistern. To reduce noise, right and left regions were combined.[44]

For the arterial-based kinetic analyses, regional [^{11}C]WAY-100635 distribution volume (DV) values were determined using both the Logan graphical method[46] and a 3-compartment model (2-tissue compartments)[47] that included a vascular volume term. A modified Logan analysis that applied generalized linear least squares smoothing to the data prior to analysis[48] was used because this effectively reduced noise-induced bias in the Logan DV as previously described for other PET radiotracers.[46] The Logan analysis was performed on PET data acquired after 25 minutes with 7 or 10 data points used for the analyses of the 60- and 90-minute data sets, respectively. Although the concentration of cerebellar 5-HT$_{1A}$ receptors is low, its influence on ROI-specific binding could not be excluded. Analyses of the cerebellar data indicated greater cerebellar DV values for a subset of recovered subjects relative to CW (see "Results" section). Therefore, the specific 5-HT$_{1A}$ receptor binding measure used in this work was one that was not strongly influenced by tissue nonspecific binding. The binding potential (BP) measure was determined as: $BP = DV_{ROI} - DV_{cerebellum}$.[47] This BP is dependent on plasma protein binding (f_1) rather than tissue free fraction (f_2).[47] As a result, plasma protein binding was measured in all subjects to determine the extent to which a group difference in [^{11}C]WAY-100635 BP could be influenced by this factor.

STATISTICAL ANALYSIS

Standard statistical software packages (SAS version 8.2 [SAS Institute Inc, Cary, NC] and StatExact 4.0 [Cytel, Cambridge, Mass]) were used for all analyses. Comparisons between CW, REC RAN, and REC BAN were made using Kruskal-Wallis tests with the exact significance levels reported. Comparisons between 2 groups were made using Wilcoxon rank sum tests. The exact levels were used because of the small sample sizes. Standard regression diagnostics were used to assess the sensitivity of the model to any observation in the data set. Pearson correlation coefficients were also computed, and exact significance levels based on Monte Carlo methods are reported.

All values are expressed as mean±SD. A significance level of $P<.05$ was selected. We adjusted for multiple comparisons using the method of false discovery rate.[49] We first applied this method to the 10 regions that were being tested for group differences (**Table 1** and **Table 2**). We also used the method of false discovery rate to adjust for the multiple comparisons for each of the 2 group comparisons and for the correlational analyses. This method was applied to each outcome, and the tests are adjusted across regions for each of the outcomes in **Table 3**. A repeated-measures analysis that used generalized estimating equations was applied to explore potential group differences in radiolabeled metabolites of [^{11}C]WAY-100635, assuming a normal distribution and an exchangeable covariance structure. Models were fit in-

Table 1. Regional [Carbonyl-^{11}C]WAY-100635 Binding Potential (BP) Between Groups—Logan Graphical Method

Region of Interest	BP, Mean ± SD			*P* Value			
	CW (n = 18)	REC BAN (n = 12)	REC RAN (n = 13)	3-Way Comparison*	CW vs REC BAN†	CW vs REC RAN†	REC BAN vs REC RAN†
Prefrontal cortex	4.17 ± 1.03	5.65 ± 0.98	4.76 ± 1.24	.004‡	<.001‡	.27	.06
Lateral orbital frontal cortex	3.87 ± 0.85	5.13 ± 0.73	4.21 ± 0.83	<.001‡	<.001‡	.32	.01‡
Medial orbital frontal cortex	4.66 ± 1.32	5.99 ± 1.02	5.19 ± 1.40	.04	.005‡	.59	.17
Supragenual cingulate	3.83 ± 0.98	5.22 ± 1.17	3.87 ± 0.95	.001‡	.001‡	.71	.001‡
Pregenual cingulate	4.52 ± 1.25	6.31 ± 1.68	4.79 ± 1.26	.02‡	.005‡	.71	.03
Subgenual cingulate	4.76 ± 1.29	6.14 ± 1.72	4.64 ± 1.43	.06	.049	.72	.03
Lateral temporal cortex	5.22 ± 1.33	6.90 ± 0.92	5.69 ± 1.30	.004‡	.002‡	.34	.02‡
Mesial temporal cortex	7.07 ± 1.96	8.62 ± 2.17	7.74 ± 2.96	.27	.11	.76	.28
Parietal cortex	3.93 ± 1.00	5.21 ± 0.93	4.27 ± 1.06	.005‡	.002‡	.49	.03‡
Dorsal raphe	2.15 ± 0.60	3.07 ± 0.71	3.00 ± 1.62	.02‡	.002‡	.27	.28

Abbreviations: CW, control women; REC BAN, women recovered from bulimia-type anorexia; REC RAN, women recovered from restricting-type anorexia.
*Group comparisons by Kruskal-Wallis test.
†Group comparisons by Wilcoxon rank sum tests.
‡Significant after adjustment for multiple comparisons. Cerebellar distribution volumes were similar between groups (*P*=.38).

Table 2. Regional [Carbonyl-^{11}C]WAY-100635 Binding Potential (BP) Between Groups—Compartmental Modeling

Region of Interest	BP, Mean ± SD			*P* Value			
	CW (n = 18)	REC BAN (n = 12)	REC RAN (n = 13)	3-Way Comparison*	CW vs REC BAN†	CW vs REC RAN†	REC BAN vs REC RAN†
Prefrontal cortex	4.07 ± 0.97	5.43 ± 0.88	4.64 ± 1.25	.005‡	.001‡	.26	.058
Lateral orbital frontal cortex	3.70 ± 0.80	4.92 ± 0.70	4.08 ± 0.85	.001‡	<.001‡	.25	.02‡
Medial orbital frontal cortex	4.47 ± 1.33	6.00 ± 1.08	5.33 ± 1.29	.01‡	.002‡	.23	.20
Supragenual cingulate	3.59 ± 0.81	4.89 ± 1.00	4.01 ± 0.99	.001‡	<.001‡	.35	.02‡
Pregenual cingulate	4.34 ± 1.23	6.01 ± 1.70	4.80 ± 1.20	.01‡	.005‡	.54	.03‡
Subgenual cingulate	4.63 ± 1.31	6.00 ± 1.47	4.64 ± 1.11	.02‡	.02‡	.92	.009‡
Lateral temporal cortex	5.10 ± 1.30	6.73 ± 0.96	5.66 ± 1.30	.002‡	<.001‡	.29	.03‡
Mesial temporal cortex	6.87 ± 2.05	9.05 ± 2.10	8.61 ± 2.75	.04‡	.02‡	.09	.58
Parietal cortex	3.84 ± 0.96	5.03 ± 0.97	4.14 ± 1.01	.007‡	.002‡	.49	.04
Dorsal raphe	2.12 ± 0.67	2.93 ± 0.62	3.27 ± 1.64	.02‡	.006‡	.07	.56

Abbreviations: See Table 1.
*Group comparisons by Kruskal-Wallis test.
†Group comparisons by Wilcoxon rank sum tests.
‡Significant after adjustment for multiple comparisons. Cerebellar distribution volumes were significantly different between groups (*P*=.02).

cluding time, group membership, and a time × group membership interaction term. Only effects with a significance level of .10 or less were retained in the model.

RESULTS

DEMOGRAPHIC VARIABLES AND BEHAVIORAL ASSESSMENTS

The REC RAN and REC BAN were of similar age as the CW (Table 3). Current body mass indexes were similar between the 3 groups; however, high lifetime body mass index was significantly lower in REC RAN compared with CW and REC BAN. As expected, low lifetime body mass index was significantly lower in REC RAN and REC BAN compared with CW. Subject groups had similar plasma β-hydroxybutyric acid values, a measure of ketone body metabolism, suggesting REC RAN and REC BAN were not in a physiologic starvation state. In addition, groups had similar plasma estradiol values. The REC RAN had the onset of their eating disorder when they were aged mean±SD 16.2±4.3 years and the REC BAN when they were aged mean±SD 16.0±3.0 years; the REC RAN and REC BAN had been recovered for mean±SD 35.0±32.3 months and 32.3±50.5 months, respectively. The REC RAN and REC BAN had, respectively, significantly higher values for eating disorder–related obsessionality (Yale-Brown-Cornell Eating Disorder Scale[55,56]), higher values for the Eating Disorder Inventory–2[51] subscales drive for thinness and bulimia, and significantly higher values in trait and state anxiety (Spielberger State-Trait Anxiety Inventory[53]), as well as significantly higher values for depression (Beck Depression Inventory[50]) and perfectionism (Frost Multidimensional Perfectionism Scale[54]) compared with CW. Only REC BAN showed higher total values for the Yale-Brown Obsessive Compulsive Scale[57,58] compared with CW, and only REC RAN had higher values for harm avoidance in comparison with CW (Table 3).

Table 3. Group Comparisons of Demographic Variables and Assessment Data

	CW (Group 1), Mean ± SD (n = 18)	REC BAN (Group 2), Mean ± SD (n = 12)	REC RAN (Group 3), Mean ± SD (n = 13)	3-Way Comparison, *P* Value*	Group Differences
Age, y	25.1 ± 5.8	28.6 ± 7.3	23.3 ± 5.2	.06	
Current BMI	22.2 ± 2.0	21.1 ± 1.8	20.8 ± 1.7	.14	
AN age at onset, y	NA	16.0 ± 3.0 (n = 11)	16.2 ± 4.3	.82†	
Duration of recovery, mo	NA	32.3 ± 50.5 (n = 11)	35.0 ± 32.3 (n = 11)	.31†	
High lifetime BMI	23.1 ± 2.0	23.7 ± 2.9	21.4 ± 2.5	.03	1, 2 > 3
Low lifetime BMI	20.1 ± 1.5	14.3 ± 1.6	13.9 ± 1.4	<.001	2, 3 < 1
Estradiol, pg/mL	51.3 ± 56.1 (n = 16)	47.8 ± 59.2	44.7 ± 41.6	.99	
β-Hydroxybutyric acid, mg/dL	7.28 ± 0.42 (n = 16)	1.04 ± 1.04	1.04 ± 1.04 (n = 11)	.56	
Depression (BDI[50])	1.0 ± 1.3 (n = 16)	6.2 ± 4.2 (n = 11)	7.1 ± 6.4 (n = 12)	<.001	2, 3 > 1
EDI-2[51] drive for thinness ("worst ever")	0.8 ± 1.4	17.0 ± 4.7 (n = 11)	16.5 ± 4.3 (n = 12)	<.001	2, 3 > 1
EDI-2 bulimia ("worst ever")	0.1 ± 0.2	8.0 ± 6.3 (n = 11)	1.7 ± 2.1 (n = 12)	<.001	2 > 3 > 1
Novelty seeking (TCI[52])	21.3 ± 5.0	22.4 ± 8.0 (n = 11)	18.7 ± 6.1 (n = 12)	.23	
Harm avoidance (TCI)	9.7 ± 3.3	12.8 ± 7.9 (n = 11)	15.9 ± 7.1 (n = 12)	.03	3 > 1
Reward dependence (TCI)	19.3 ± 2.6	18.5 ± 3.1 (n = 11)	16.7 ± 3.7 (n = 12)	.12	
State anxiety (STAI[53])	25.6 ± 5.2	33.9 ± 8.5 (n = 11)	35.3 ± 11.8 (n = 12)	.004	2, 3 > 1
Trait anxiety (STAI)	26.8 ± 5.2	37.5 ± 9.5 (n = 11)	36.3 ± 10.0 (n = 12)	.001	2, 3 > 1
Perfectionism (MPS[54])	54.6 ± 11.4	105.5 ± 23.1 (n = 11)	99.0 ± 18.4 (n = 12)	<.001	2, 3 > 1
Yale-Brown-Cornell Eating Disorders Scale[55,56]	0.3 ± 0.8	4.1 ± 5.1 (n = 10)	7.0 ± 7.6 (n = 12)	.001	2, 3 > 1
Yale-Brown Obsessive-Compulsive Scale[51,58]	0.4 ± 1.4	9.8 ± 10.4 (n = 10)	4.3 ± 6.3 (n = 12)	.02	2 > 1

Abbreviations: AN, anorexia nervosa; BDI, Beck Depression Inventory; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); CW, control women; EDI-2, Eating Disorder Inventory–2; MPS, Multidimensional Perfectionism Scale; NA, not applicable; REC BAN, women recovered from bulimia-type anorexia; REC RAN, women recovered from restricting-type anorexia; STAI, State-Trait Anxiety Inventory; TCI, Temperament and Character Inventory.

SI factor conversions: To convert estradiol to picomoles per liter, multiply by 3.671; β-hydroxybutyric acid to micromoles per liter, 96.05.

*Group comparison by Kruskal-Wallis test.

†Group comparison by Wilcoxon rank sum test.

PET DATA

Plasma Data

The repeated-measures analysis of the unmetabolized fraction of [^{11}C]WAY-100635 (8 points) indicated that the REC RAN group was different from the other 2 groups. Both the group (P=.05) and the group × time interaction (P=.08) were of marginal significance in this model. Coefficients associated with group indicated a higher fraction of unmetabolized [^{11}C]WAY-100635 across time for REC RAN while the interaction term was negative indicating a decline across time in this group as well. However, this decline across time was observed in REC BAN and CW as well (**Figure 1**). No significant differences in plasma free fraction (f_1) were found between REC RAN (mean±SD f_1, 0.088±0.028), REC BAN (mean±SD f_1, 0.100±0.040), and CW (mean±SD f_1, 0.093±0.030) (P=.79), from whom these data were available.

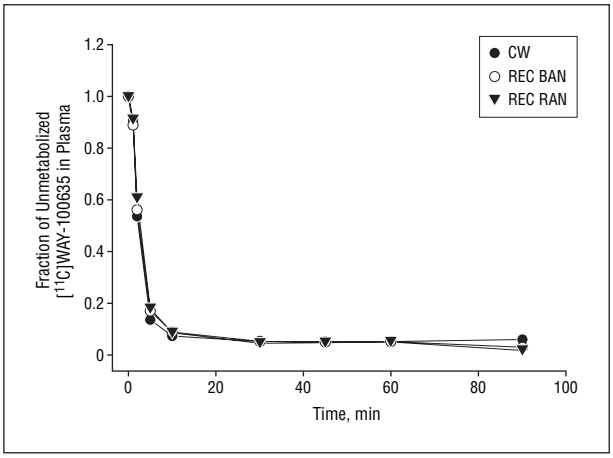

Figure 1. Fraction of unmetabolized [carbonyl-^{11}C]WAY-100635 in plasma across time. CW indicates control women; REC RAN, women recovered from restricting-type anorexia; REC BAN, women recovered from bulimia-type anorexia.

ROI-Based Analysis: Logan Graphical Method

The cerebellar DV values were similar (P=.38; 3 groups) but somewhat lower for CW (mean±SD, 0.68±0.15) than for REC RAN (mean±SD, 0.79±0.26) and REC BAN (mean±SD, 0.78±0.15). The regional [^{11}C]WAY-100635 BP values followed the known rank order of 5-HT_{1A} receptor binding,[59,60] as presented in Table 1. After adjustment for multiple comparisons, we found significant differences for [^{11}C]WAY-100635 BP values among the 3 groups for the prefrontal, lateral orbital frontal, lateral temporal, parietal cortex, supragenual and pregenual cingulate, and dorsal raphe. Illustrative scatterplots (**Figure 2**) are shown for the lateral orbital frontal cortex and the dorsal raphe. Further analysis revealed that only REC BAN had a highly significant increase of [^{11}C]WAY-100635 BP compared with CW in prefrontal, lateral and medial orbital frontal, lateral temporal, parietal, and supragenual and pregenual cingulate regions as well as in the dorsal raphe, after adjustment for mul-

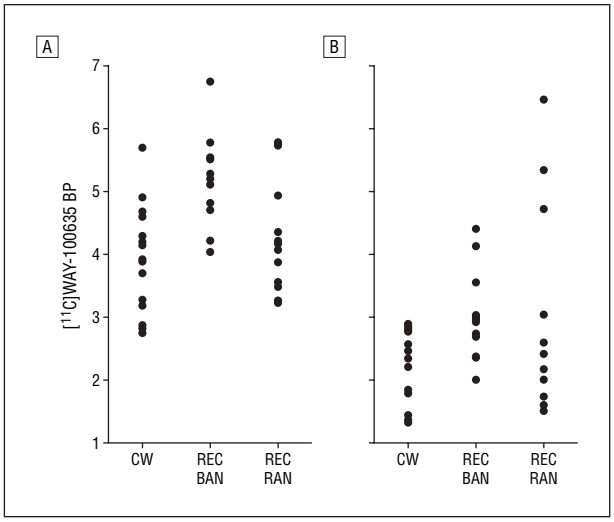

Figure 2. Scatter histograms of the [carbonyl-^{11}C]WAY-100635 ([^{11}C]WAY-100635) binding potential (BP) values for control women (CW), women recovered from bulimia-type anorexia (REC BAN), and women recovered from restricting-type anorexia (REC RAN) in the lateral orbital frontal cortex (A) and the dorsal raphe (B).

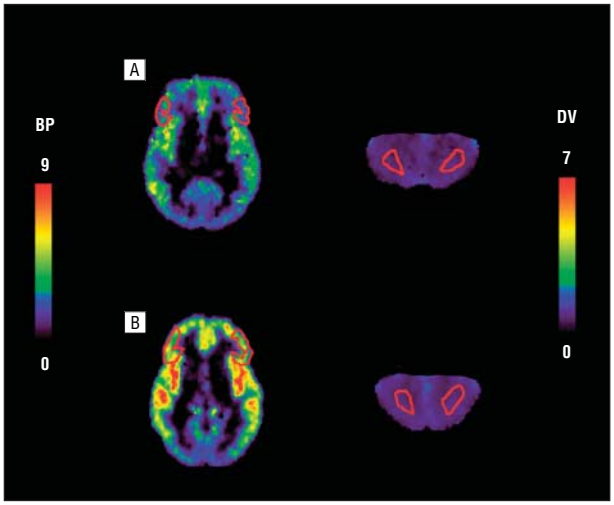

Figure 3. Parametric binding potential (BP) images (left panel) and reference region distribution volume ($DV_{cerebellum\ (CER)}$) images (right panel) calculated from [carbonyl-^{11}C]WAY-100635 ([^{11}C]WAY-100635) positron emission tomography data acquired in 1 control woman (A) and 1 individual recovered from bulimia-type anorexia nervosa (B). A voxel-based Logan analysis was used to create the BP images ($BP = DV_{region\ of\ interest\ (ROI)} - DV_{CER}$). The red line in the left panel depicts the lateral orbital frontal cortex region of interest, and the red line in the right panel depicts the cerebellar region of interest.

tiple comparisons. **Figure 3** depicts our finding of significantly increased [^{11}C]WAY-100635 BP in the lateral orbital frontal cortex of REC BAN relative to CW. The REC RAN did not differ significantly from the CW in any of the assessed regions. The REC BAN had significantly increased [^{11}C]WAY-100635 BP in the lateral orbital frontal, lateral temporal, and parietal cortex, as well as in the supragenual cingulate compared with REC RAN.

The temporal stability of the outcome measures was examined in the subset of subjects (11 CW, 6 REC BAN, 4 REC RAN) for which a full 90-minute emission data set was available. High correlations were observed between the cerebellar DV and regional BP measures calculated using the 60-minute and 90-minute data sets (CW, $r = 0.96$-0.99; REC RAN and REC BAN, $r = 0.98$-0.99). The 90-minute values generally exceeded the 60-minute values. As an example, the mesial temporal cortex 90-minute BP values were about 15% greater than the 60-minute values (**Figure 4**).

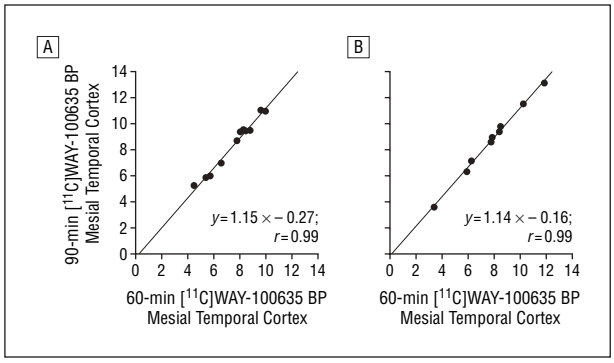

Figure 4. Relationship of 60-minute and 90-minute data of [carbonyl-^{11}C]WAY-100635 ([^{11}C]WAY-100635) binding potential (BP) in the mesial temporal cortex in control women (A) and in the combined group of women recovered from bulimia-type and restricting-type anorexia (B). *r* indicates Pearson correlation coefficient.

ROI-Based Analysis: Compartmental Modeling

The compartmental cerebellar DV values differed between groups ($P = .02$; 3 groups). Higher cerebellar DV was found for REC BAN (mean ± SD, 0.65 ± 0.12) vs CW (mean ± SD, 0.53 ± 0.12) with $P = .01$ and was only higher at a trend level for cerebellar DV of REC BAN vs REC RAN (mean ± SD, 0.60 ± 0.23) with $P = .05$. In 1 CW, the cerebellar k_4 value was negative. A 2-compartment, 3-parameter model fit to this subject's data yielded a 2-compartment cerebellar DV value that was within 1% agreement of the 3-compartment DV value. Group differences in the regional [^{11}C]WAY-100635 BP values were similar for the compartmental (Table 2) and Logan analyses (Table 1), with additional group differences of higher [^{11}C]WAY-100635 BP for mesial temporal and subgenual cingulate regions of REC BAN (relative to CW). Good agreement was found between the compartmental and Logan results. Therefore, comparisons of receptor binding and behavioral measures were performed using only 1 BP measure determined using the simpler Logan method.

RELATIONSHIP OF DEMOGRAPHIC AND BEHAVIORAL DATA WITH [^{11}C]WAY-100635 BP

Seventeen subjects in the REC RAN and REC BAN groups had a history of major depressive disorder and 12 subjects had a history of obsessive-compulsive disorder. Two subjects fulfilled criteria for social phobia, 2 had a lifetime diagnosis of panic disorder, 4 fulfilled criteria for specific phobia, and 4 subjects had a lifetime posttraumatic stress disorder diagnosis. Additional lifetime comorbidity included alcohol dependence (1 subject), alcohol abuse (1 subject), cocaine abuse (1 subject), and opioid dependence (2 subjects). Seven REC BAN had a

history of bulimia nervosa (BN). The number of individuals with any kind of comorbid Axis I disorder was similar between REC BAN and REC RAN. None of the REC RAN and REC BAN had a history of any psychotic disorder. Subjects with comorbid obsessive-compulsive disorder, major depressive disorder, any anxiety or substance use disorder, or history of BN did not differ in [^{11}C]WAY-100635 BP from those subjects without obsessive-compulsive disorder, major depressive disorder, anxiety or substance use disorder, or history of BN, within each diagnostic group. No relationships were found for either group between [^{11}C]WAY-100635 BP and age, current body mass index, plasma β-hydroxybutyric acid value, estradiol value, or duration of recovery.

The REC RAN showed a positive relationship between harm avoidance and [^{11}C]WAY-100635 BP in the mesial temporal cortex (r=0.83; P=.001) and subgenual cingulate (r=0.66; P=.03) (**Figure 5**) as well as positive relationships at a trend to significance in lateral temporal (r=0.54; P=.07), medial orbital frontal (r=0.57; P=.05), and parietal cortices (r=0.52; P=.07). After adjustment for multiple comparisons, only the positive relationship in the mesial temporal cortex remained significant. There was no relationship between harm avoidance and [^{11}C]WAY-100635 BP for CW or REC BAN or for the group of REC RAN and REC BAN considered together.

Ten CW, 7 REC RAN, and 5 REC BAN were taking birth control pills at the time of the study. There were no differences in [^{11}C]WAY-100635 BP across ROIs between subjects who were or were not taking birth control pills within each group. Furthermore, there was no correlation between day of follicular phase (day of menses) and [^{11}C]WAY-100635 BP in any of the groups.

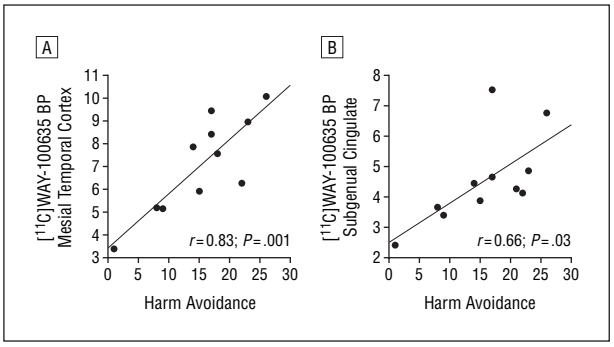

Figure 5. Correlation of harm avoidance and [carbonyl-^{11}C]WAY-100635 ([^{11}C]WAY-100635) binding potential (BP) in the mesial temporal cortex (A) and subgenual cingulate (B) in women recovered from restricting-type anorexia. *r* indicates Pearson correlation coefficient.

COMMENT

This study provides further evidence that a disturbance of 5-HT neuronal function and behavioral symptoms persist after normalization of weight and nutritional status in AN. The REC BAN had a 22% to 43% increase in dorsal raphe and cortical [^{11}C]WAY-100635 BP, suggesting they had elevated autosynaptic and postsynaptic 5-HT$_{1A}$ receptor activity. In contrast, REC RAN had normal [^{11}C]WAY-100635 BP values, which was associated with harm avoidance.

DIFFERENCES BETWEEN RAN AND BAN

Subjects with RAN and BAN have similarities and differences in 5-HT neuronal function. For example, REC RAN, REC BAN, and individuals recovered from BN have increased baseline cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations,[33,61] the major metabolite of brain 5-HT, which presumably indicates increased extracellular 5-HT concentrations. However, individuals with RAN and BAN have differences in accumulations of cerebrospinal fluid 5-hydroxyindoleacetic acid after administration of probenecid, which blocks the exit of acid metabolites from cerebrospinal fluid.[62] Moreover, individuals recovered from BN,[63] REC RAN[40] (5 CW and 7 REC RAN were in this study), and REC BAN[41] (8 CW and 6 REC BAN were in this study) have reduced [fluoro-^{18}F]altanserin BP in PET studies, suggesting they have reduced 5-HT$_{2A}$ receptor activity. Reduced 5-HT$_{2A}$ activity would be an expected compensatory down-regulation response to increased extracellular 5-HT concentrations.

In comparison, increased activity of the 5-HT$_{1A}$ receptor function may only be associated with binge-and-purge symptoms. In support of this possibility, increased postsynaptic 5-HT$_{1A}$ activity has been reported in ill subjects with BN[64] who never had a history of AN. Moreover, our laboratory (S.E.H., U.F.B., G.K.F., C.C.M., J.C.P., C.A.M., A.W., and W.H.K., unpublished data, July 2004) found elevated [^{11}C]WAY-100635 BP in individuals recovered from BN without a history of AN. Other studies have noted that individuals with RAN and BAN have differences in 5-HT metabolism. Recent PET studies from our laboratory (U.F.B., G.K.F., S.E.H., J.C.P., C.C.M., L.W., C.A.M., A.W., J.H., S.K.Z., C.W.M., and W.H.K., unpublished data, June 2005) show that individuals recovered from BAN have reduced 5-HT transporter activity compared with subjects with RAN. These latter data raise the possibility that individuals with BAN have a relative increase of extracellular 5-HT compared with subjects with RAN. As discussed later, increased presynaptic and postsynaptic 5-HT$_{1A}$ receptor activity may serve to counteract increased extracellular 5-HT.[65]

Receptor and transporter activity and 5-hydroxyindoleacetic acid concentrations reflect the complex dynamics of 5-HT pathways because other 5-HT receptors, intracellular messengers, and influences of other neurotransmitter systems are involved. The data described earlier can be interpreted to suggest that a dysregulation of 5-HT neuronal pathways persists after recovery from an eating disorder. Most importantly, it is possible that each eating disorder subtype has different patterns of dysregulation of components of this pathway, which may help explain why differences in symptoms occur in subtypes.

In comparison, decreased autosynaptic and postsynaptic [^{11}C]WAY-100635 BP have been found in depression in the ill[45,66,67] and recovered state[68] and in panic disorder.[69] Bulimia nervosa is often comorbid with depression or panic disorders because these disorders may affect the same pathways but have different loci of pathophysiology within those pathways.

GENERALIZED 5-HT$_{1A}$ RECEPTOR FINDINGS IN RELATIONSHIP TO 5-HT NEURONAL FUNCTION

Our study found that increased [^{11}C]WAY-100635 BP in REC BAN was generalized to all regions sampled. Moreover, significant positive relationships were seen for [^{11}C]WAY-100635 BP for the dorsal raphe 5-HT$_{1A}$ autoreceptors and postsynaptic 5-HT$_{1A}$ receptors. In addition, the cortical [^{11}C]WAY-100635 BP values were significantly positively related to each other (data not shown). Other PET studies show similar positive correlations for [^{11}C]WAY-100635 BP in healthy subjects[70] and depression.[66,67]

The 5-HT$_{1A}$ receptor is both an autoreceptor in the raphe nucleus and a postsynaptic receptor in the forebrain. Yet these data suggest that alterations in the 5-HT$_{1A}$ receptor system seem to occur in the same direction. For example, both autoreceptors and postsynaptic receptors were increased in the individuals recovered from BAN and decreased in depression.[66,67] Does this make physiological sense? Autoreceptor 5-HT$_{1A}$ activation is thought to be a negative feedback mechanism that reduces raphe activity.[71] Recent studies show that postsynaptic 5-HT$_{1A}$ receptors in frontal regions have inhibitory properties on raphe activity[72] through feedback loops, although less is known about postsynaptic 5-HT$_{1A}$ feedback to the raphe in other brain regions. In summary, if both autoreceptor and postsynaptic 5-HT$_{1A}$ receptor activity serve to inhibit raphe activity, then it makes sense that both 5-HT$_{1A}$ autoreceptors and postsynaptic receptors have similar levels of activity in the brain.

HARM AVOIDANCE AND REGIONAL FINDINGS

Only REC RAN had positive relationships between harm avoidance and postsynaptic [^{11}C]WAY-100635 BP in the subgenual cingulate, mesial temporal, lateral temporal, medial orbital frontal, and parietal cortex. Such correlations were not found in REC BAN. Other studies from our group[41] found that REC BAN had positive relationships between harm avoidance and [^{18}F]altanserin BP in the left subgenual cingulate, left lateral temporal, and mesial temporal cortex. Such relationships were not found in REC RAN.[40] Together, these studies raise the possibility that cingulate and temporal regions may play a role in elevated harm avoidance in people with eating disorders.

Recent data raise the possibility that the interaction and balance between 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor activity may be important. Postsynaptic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors are colocalized and interact to mediate, respectively, the direct hyperpolarizing and depolarizing actions of 5-HT on cortical neurons,[65] which in turn project to numerous cortical and subcortical areas. Thus, a balance between postsynaptic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor activity on neurons may modulate the descending excitatory input into limbic and motor structures.[73] While REC RAN and REC BAN may have differences in 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor activity, the balance between these 2 receptors (eg, relatively more 5-HT$_{1A}$ than 5-HT$_{2A}$ receptor activity) appears to be similar for individuals with RAN and BAN (U.F.B., S.E.H., G.K.F., C.C.M., J.C.P., C.A.M., A.W., and W.H.K., unpublished data, July 2004), and this balance may be altered compared with CW. Other data from our laboratory (W.H.K., U.F.B., G.K.F., S.E.H., J.C.P., C.C.M., L.W., C.W.M., A.W., C.A.M., J.H., and S.K.Z., unpublished data, March 2005) suggest that the balance of postsynaptic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors contributes to modulating behavior inhibition in CW.

Could alterations of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor interactions be related to symptoms of harm avoidance in cingulate-temporal regions? Harm avoidance is common in AN.[74,75] Moreover, most individuals with eating disorders have anxious as well as obsessive-compulsive behaviors, which often start in childhood before the onset of their eating disorder.[76,77] The anterior cingulate, a region that normally serves to inhibit amygdala reactivity,[78] also has connections to the periaqueductal gray. 5-HT$_{1A}$ and 5-HT$_{2A}$ in this region contribute to the modulation of escape behavior in rats, a defensive behavior that has been related to panic disorder.[79] The subgenual cingulate is involved in conditioned emotional learning, vocalizations associated with expressing internal states, and assigning emotional valence to internal and external stimuli.[80-82] It is thought to have a role in emotional and autonomic response as well as in regulating overall serotonergic activity[83] and has been implicated in depression.[66,84-86] Mood disorders are common in eating disorders,[3] as are disturbances of energy metabolism.[87] The subgenual cingulate has extensive connections with the amygdala, periaqueductal gray, frontal lobes, ventral striatum, and autonomic brainstem nuclei. Mesial temporal regions include the amygdala and related regions, which play a pivotal role in anxiety and fear as well as in the modulation and integration of cognition and mood. The amygdala may enable the individual to initiate adaptive behaviors to threat based on the nature of the threat and prior experience.[88] Finally, increased 5-HT$_{1A}$ receptor activity may inhibit function of the anterior cingulate, a region that normally serves to inhibit amygdala reactivity,[78] thus contributing to heightened amygdala response. In summary, these data raise the speculation that 5-HT-related alterations of subgenual cingulate and amygdala regions contribute to anxious behaviors in AN.

RELATIONSHIP TO AGE

We did not find correlations of age and 5-HT$_{1A}$ receptor binding (using the 60-minute or 90-minute data set). In comparison, some postmortem and in vivo studies[22,44,89,90] have shown aging reductions in 5-HT$_{1A}$ receptor densities whereas others did not find an age-dependent decline in 5-HT$_{1A}$ receptors.[70,91,92] The age range of our subjects was narrow. In addition, all of our subjects were female. Other studies[44] have raised the possibility of relationships between regional [^{11}C]WAY-100635 BP and age in men.

LIMITATIONS

We relied on subject self-report of recovered status. However, normal plasma β-hydroxybutyric acid and estradiol values in REC RAN and REC BAN support the prob-

ability that they have normal nutritional and gonadal status. Another issue of concern was whether the observed differences in the cerebellar DV could be accounted for by group differences in the level of [^{11}C]WAY-100635 in plasma. The Logan graphical method did not correct for blood volume while the compartmental model included a vascular volume term. The cerebellar DV difference was only statistically significant for the compartmental method, which accounted for larger vascular volume terms for REC BAN (P=.02) as well as REC RAN (P=.01), relative to CW (data not shown). It is therefore unlikely that differences in vascular volume fully accounted for the group difference in cerebellar DV. It is also not likely that the difference resulted from differences in the levels of radiolabeled metabolites or protein binding because these parameters were similar between the groups. It is not known why the [^{11}C]WAY-100635 cerebellar DV would be increased in REC BAN since the cerebellum has been thought to be relatively devoid of 5-HT$_{1A}$ receptors.[60] Limited evidence suggests that the cerebellum is populated by 5-HT$_{1A}$ receptors in the neonatal period that disappear during early childhood. One study raised the possibility that cerebellar 5-HT$_{1A}$ receptor activity persists in the cerebellum in patients with schizophrenia.[93] Whether REC BAN have persistent cerebellar 5-HT$_{1A}$ receptors is not known.

The BP measure in this study was influenced by plasma nonspecific binding. As described earlier, no group difference was detected between the mean [^{11}C]WAY-100635 f_1 measures. The individual BP values were, therefore, not corrected by the plasma protein binding value because such a correction would introduce variability into the BP values because f_1 is a somewhat variable measure. Another concern with this choice of BP is that the low cerebellar DV value would be irrelevant in an absolute sense, relative to the larger regional DV value. For comparison, specific binding was also computed using the DV ratio (dependent on f_2), and we found similar group differences but at a trend level (data not shown).

[^{11}C]WAY-100635 PET studies in humans have acquired data across 60 minutes.[66,94,95] Parsey et al[47] reported that regional [^{11}C]WAY-100635 binding measures were stable in most brain areas when data were acquired across 80 to 90 minutes, although 110 to 120 minutes was indicated for the dorsal raphe. In our investigation, earlier studies were acquired across 60 minutes but later studies were performed across 90 minutes. Linear regression of the regional [^{11}C]WAY-100635 BP measures determined for the 60-minute and 90-minute data sets yielded highly positive correlations, supporting the validity of the results obtained using the larger 60-minute data set. Furthermore, similar trends in the group differences were observed for the 90-minute and 60-minute data sets (data not shown).

Only REC BAN were found to have significant increases of [^{11}C]WAY-100635 BP relative to CW. The REC RAN also had increased [^{11}C]WAY-100635 BP in some ROIs compared with CW, but no difference reached statistical significance. The sample size in this study may have lacked power. A power computation for the comparison of CW and REC RAN revealed that the difference between the 2 groups must be of the order of 1 SD, assuming a 2-sided t test was performed at a significance level of P=.05 and a power of 0.80. This was the case for CW vs REC BAN, but replications of these findings in larger samples are needed.

SUMMARY

In summary, this study lends further credibility to the possibility that women with AN have a persistent disturbance of 5-HT neuronal systems that may be related to increased anxiety. While it cannot be certain whether 5-HT alterations are a "scar" following cessation of low weight and malnutrition, the fact that premorbid anxiety disorders occur in AN supports the possibility that altered 5-HT pathway function could predate the onset of AN and persist after recovery. There are no proven treatments for AN, and this illness has the highest mortality of any psychiatric disorder.[96] These data offer the promise of a new understanding of the pathogenesis of AN and new drug and psychological treatment targets.

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REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. Washington, DC: American Psychiatric Association; 1994.
2. Kendler KS, Walters EE, Neale MC, Kessler RC, Heath AC, Eaves LJ. The structure of the genetic and environmental risk factors for six major psychiatric disorders in women: phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism. *Arch Gen Psychiatry*. 1995;52:374-383.
3. Lilenfeld LR, Kaye WH, Greeno CG, Merikangas KR, Plotnicov K, Pollice C, Rao R, Strober M, Bulik CM, Nagy L. A controlled family study of anorexia nervosa and bulimia nervosa: psychiatric disorders in first-degree relatives and effects of proband comorbidity. *Arch Gen Psychiatry*. 1998;55:603-610.
4. Strober M, Freeman R, Lampert C, Diamond J, Kaye W. Controlled family study of anorexia nervosa and bulimia nervosa: evidence of shared liability and transmission of partial syndromes. *Am J Psychiatry*. 2000;157:393-401.
5. Blundell JE. Serotonin and appetite. *Neuropharmacology*. 1984;23:1537-1551.
6. Leibowitz SF, Shor-Posner G. Brain serotonin and eating behavior. *Appetite*. 1986; 7(suppl):1-14.
7. Cloninger CR. A systematic method for clinical description and classification of personality variants: a proposal. *Arch Gen Psychiatry*. 1987;44:573-588.
8. Barr LC, Goodman WK, Price LH, McDougle CJ, Charney DS. The serotonin hypothesis of obsessive compulsive disorder: implications of pharmacologic challenge studies. *J Clin Psychiatry*. 1992;53(suppl):17-28.
9. Higley JD, Linnoila M. Low central nervous system serotonergic activity is trait-like and correlates with impulsive behavior: a nonhuman primate model investigating genetic and environmental influences on neurotransmission. *Ann N Y Acad Sci*. 1997;836:39-56.
10. Kaye WH. Anorexia and bulimia nervosa, obsessional behavior, and serotonin. In: Kaye WH, Jimerson DC, eds. *Eating Disorders*. London, England: Balliere's Tindell, Inc; 1997:319-337.
11. Lucki I. The spectrum of behaviors influenced by serotonin. *Biol Psychiatry*. 1998; 44:151-162.
12. Mann JJ. Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. *Neuropsychopharmacology*. 1999;21(2 suppl): 99S-105S.
13. Kaye WH, Gwirtsman HE, George DT, Jimerson DC, Ebert MH. CSF 5-HIAA concentrations in anorexia nervosa: reduced values in underweight subjects normalize after weight gain. *Biol Psychiatry*. 1988;23:102-105.
14. Brewerton TD, Jimerson DC. Studies of serotonin function in anorexia nervosa. *Psychiatry Res*. 1996;62:31-42.
15. Wolfe BE, Metzger ED, Jimerson DC. Research update on serotonin function in bulimia nervosa and anorexia nervosa. *Psychopharmacol Bull*. 1997;33:345-354.
16. Walsh BT, Devlin MJ. Eating disorders: progress and problems. *Science*. 1998; 280:1387-1390.
17. Kaye WH, Nagata T, Weltzin TE, Hsu LK, Sokol MS, McConaha C, Plotnicov KH, Weise J, Deep D. Double-blind placebo-controlled administration of fluoxetine in restricting- and restricting-purging-type anorexia nervosa. *Biol Psychiatry*. 2001; 49:644-652.
18. Cervo L, Mocaer E, Bertaglia A, Samanin R. Roles of 5-HT_{1A} receptors in the dorsal raphe and dorsal hippocampus in anxiety assessed by the behavioral effects of 8-OH-DPAT and S 15535 in a modified Geller-Seifter conflict model. *Neuropharmacology*. 2000;39:1037-1043.
19. File SE, Kenny PJ, Cheeta S. The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. *Pharmacol Biochem Behav*. 2000;66:65-72.
20. Olivier B, Pattij T, Wood S, Oosting R, Sarnyai Z, Toth M. The 5-HT_{1A} receptor knockout mouse and anxiety. *Behav Pharmacol*. 2001;12:439-450.
21. Matsubara S, Arora RC, Meltzer HY. Serotonergic measures in suicide brain: 5-HT1A binding sites in frontal cortex of suicide victims. *J Neural Transm Gen Sect*. 1991; 85:181-194.
22. Arango V, Underwood MD, Gubbi AV, Mann JJ. Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims. *Brain Res*. 1995;688:121-133.
23. Collin M, Backberg M, Onnestam K, Meister B. 5-HT1A receptor immunoreactivity in hypothalamic neurons involved in body weight control. *Neuroreport*. 2002; 13:945-951.
24. Gur E, Newman M, Avraham Y, Dremencov E, Berry E. The differential effects of food restriction on 5-HT1A and 5-HT1B receptor mediated control of serotonergic transmission in the hippocampus and hypothalamus of rats. *Nutr Neurosci*. 2003;6:169-175.
25. Currie P, Braver M, Mirza A, Sricharoon K. Sex differences in the reversal of fluoxetine-induced anorexia following raphe injections of 8-OH-DPAT. *Psychopharmacology (Berl)*. 2004;172:359-364.
26. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology*. 1999;38:1083-1152.
27. Stahl SM. Mechanism of action of serotonin selective reuptake inhibitors: serotonin receptors and pathways mediate therapeutic effects and side effects. *J Affect Disord*. 1998;51:215-235.
28. Gordon I, Lask B, Bryant-Waugh R, Christie D, Timimi S. Childhood-onset anorexia nervosa: towards identifying a biological substrate. *Int J Eat Disord*. 1997; 22:159-165.
29. Ellison AR, Fong J. Neuroimaging in eating disorders. In: Hoek HW, Treasure JL, Katzman MA, eds. *Neurobiology in the Treatment of Eating Disorders*. Chichester, England: John Wiley & Sons Ltd; 1998:255-269.
30. Gordon CM, Dougherty DD, Fischman AJ, Emans SJ, Grace E, Lamm R, Alpert NM, Majzoub JA, Rausch SL. Neural substrates of anorexia nervosa: a behavioral challenge study with positron emission tomography. *J Pediatr*. 2001; 139:51-57.
31. Saudou F, Hen R. 5-Hydroxytryptamine receptor subtypes in vertebrates and invertebrates. *Neurochem Int*. 1994;25:503-532.
32. Burnet PW, Eastwood SL, Harrison PJ. [3H]WAY-100635 for 5-HT1A receptor autoradiography in human brain: a comparison with [3H]8-OH-DPAT and demonstration of increased binding in the frontal cortex in schizophrenia. *Neurochem Int*. 1997;30:565-574.
33. Kaye WH, Gwirtsman HE, George DT, Ebert MH. Altered serotonin activity in anorexia nervosa after long-term weight restoration: does elevated cerebrospinal fluid 5-hydroxyindoleacetic acid level correlate with rigid and obsessive behavior? *Arch Gen Psychiatry*. 1991;48:556-562.
34. O'Dwyer AM, Lucey JV, Russell GF. Serotonin activity in anorexia nervosa after long-term weight restoration: response to D-fenfluramine challenge. *Psychol Med*. 1996;26:353-359.
35. Ward A, Brown N, Lightman S, Campbell IC, Treasure J. Neuroendocrine, appetitive and behavioural responses to d-fenfluramine in women recovered from anorexia nervosa. *Br J Psychiatry*. 1998;172:351-358.
36. Casper RC. Personality features of women with good outcome from restricting anorexia nervosa. *Psychosom Med*. 1990;52:156-170.
37. Deep AL, Nagy LM, Weltzin TE, Rao R, Kaye WH. Premorbid onset of psychopathology in long-term recovered anorexia nervosa. *Int J Eat Disord*. 1995; 17:291-297.
38. Srinivasagam NM, Kaye WH, Plotnicov KH, Greeno C, Weltzin TE, Rao R. Persistent perfectionism, symmetry, and exactness after long-term recovery from anorexia nervosa. *Am J Psychiatry*. 1995;152:1630-1634.
39. Bulik CM, Sullivan PF, Fear JL, Joyce PR. Eating disorders and antecedent anxiety disorders: a controlled study. *Acta Psychiatr Scand*. 1997;96:101-107.
40. Frank GK, Kaye WH, Meltzer CC, Price JC, Greer P, McConaha C, Skovira K. Reduced 5-HT2A receptor binding after recovery from anorexia nervosa. *Biol Psychiatry*. 2002;52:896-906.
41. Bailer UF, Price JC, Meltzer CC, Mathis CA, Frank GK, Weissfeld L, McConaha CW, Henry SE, Brooks-Achenbach S, Barbarich NC, Kaye WH. Altered 5-HT_{2A} receptor binding after recovery from bulimia-type anorexia nervosa: relationships to harm avoidance and drive for thinness. *Neuropsychopharmacology*. 2004; 29:1143-1155.
42. Meltzer CC, Price J, Mathis C, Butters M, Ziolko S, Moses-Kolko E, Mazumdar S, Mulsant B, Houck PR, Lopresti B, Weissfeld L, Reynolds C. Serotonin 1A receptor binding and treatment responses in late-life depression. *Neuropsychopharmacology*. 2004;29:2258-2265.
43. McCarron JA, Turton D, Pike VW, Poole K. Remotely controlled production of the 5-HT1A receptor radioligand, [carbonyl-11C]WAY-100635, via 11C-carboxylation of an immobilized Grignard reagent. *J Labelled Comp Radiopharm*. 1996;38:941-953.
44. Cidis Meltzer C, Drevets W, Price J, Mathis C, Lopresti B, Greer P, Villemagne V, Holt D, Mason N, Houck P, Reynolds C, DeKosky S. Gender-specific aging effects on the serotonin 1A receptor. *Brain Res*. 2001;895:9-17.
45. Drevets WC, Frank E, Price JC, Kupfer DJ, Greer PJ, Mathis C. Serotonin type-1A receptor imaging in depression. *Nucl Med Biol*. 2000;27:499-507.
46. Logan J, Fowler J, Volkow N, Ding Y, Wang G, Alexoff D. A strategy for removing the bias in the graphical analysis method. *J Cereb Blood Flow Metab*. 2001; 21:307-320.
47. Parsey RV, Slifstein M, Hwang DR, Abi-Dargham A, Simpson N, Mawlawi O, Guo NN, Van Heertum R, Mann JJ, Laruelle M. Validation and reproducibility of measurement of 5-HT_{1A} receptor parameters with [carbonyl-^{11}C]WAY-100635 in humans: comparison of arterial and reference tissue input functions. *J Cereb Blood Flow Metab*. 2000;20:1111-1133.
48. Price J, Xu L, Mazumdar S, Meltzer C, Drevets W, Mathis C, Kelley D, Ryan C, Reynolds C. Impact of graphical analysis bias on group comparisons of regional [carbonyl-11C]WAY binding potential measures [abstract]. *Neuroimage*. 2002;16:S72.
49. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc B*. 1995;57:289-300.

50. Beck AT, Ward M, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4:561-571.
51. Garner DM. *Eating Disorder Inventory-2 Professional Manual*. Lutz, Fla: Psychological Assessment Resources, Inc; 1990.
52. Cloninger CR, Przybeck TR, Svrakic DM, Wetzel RD. *The Temperament and Character Inventory (TCI): A Guide to Its Development and Use*. St Louis, Mo: Center for Psychobiology of Personality, Washington University; 1994.
53. Spielberger CD, Gorsuch RL, Lushene RE. *STAI Manual for the State-Trait Anxiety Inventory*. Palo Alto, Calif: Consulting Psychologists Press; 1970.
54. Frost RO, Marten P, Lahart C, Rosenblate R. The dimensions of perfectionism. *Cognit Ther Res*. 1990;14:449-468.
55. Mazure CM, Halmi KA, Sunday SR, Romano SJ, Einhorn AM. The Yale-Brown-Cornell Eating Disorder Scale: development, use, reliability and validity. *J Psychiatr Res*. 1994;28:425-445.
56. Sunday SR, Halmi KA, Einhorn A. The Yale-Brown-Cornell Eating Disorder Scale: a new scale to assess eating disorder symptomatology. *Int J Eat Disord*. 1995; 18:237-245.
57. Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, Heninger GR, Charney DS. The Yale-Brown Obsessive Compulsive Scale, I: development, use, and reliability. *Arch Gen Psychiatry*. 1989;46:1006-1011.
58. Goodman WK, Price LH, Rasmussen SA, Mazure C, Delgado P, Heninger GR, Charney DS. The Yale-Brown Obsessive Compulsive Scale, II: validity. *Arch Gen Psychiatry*. 1989;46:1012-1016.
59. Hall H, Lundkvist C, Halldin C, Farde L, Pike VW, McCarron JA, Fletcher A, Cliffe IA, Barf T, Wikstrom H, Sedvall G. Autoradiographic localization of 5-HT_{1A} receptors in the post-mortem human brain using [^{3}H]WAY-100635 and [^{11}C]way-100635. *Brain Res*. 1997;745:96-108.
60. Pazos A, Palacios JM. Quantitative autoradiographic mapping of serotonin receptors in the rat brain, I: serotonin-1 receptors. *Brain Res*. 1985;346:205-230.
61. Kaye WH, Greeno CG, Moss H, Fernstrom J, Fernstrom M, Lilenfeld LR, Weltzin TE, Mann JJ. Alterations in serotonin activity and psychiatric symptomatology after recovery from bulimia nervosa. *Arch Gen Psychiatry*. 1998;55: 927-935.
62. Kaye WH, Ebert MH, Gwirtsman HE, Weiss SR. Differences in brain serotonergic metabolism between nonbulimic and bulimic patients with anorexia nervosa. *Am J Psychiatry*. 1984;141:1598-1601.
63. Kaye WH, Frank GK, Meltzer CC, Price JC, McConaha CW, Crossan PJ, Klump KL, Rhodes L. Altered serotonin 2A receptor activity in women who have recovered from bulimia nervosa. *Am J Psychiatry*. 2001;158:1152-1155.
64. Tiihonen J, Keski-Rahkonen A, Lopponen M, Muhonen M, Kajander J, Allonen T, Nagren K, Hietala J, Rissanen A. Brain serotonin 1A receptor binding in bulimia nervosa. *Biol Psychiatry*. 2004;55:871-873.
65. Santana N, Bortolozzi A, Serrats J, Mengod G, Artigas F. Expression of $serotonin_{1A}$ and $serotonin_{2A}$ receptor in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb Cortex*. 2004;14:1100-1009.
66. Drevets WC, Frank E, Price JC, Kupfer DJ, Holt D, Greer PJ, Huang Y, Gautier C, Mathis C. PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry*. 1999;46:1375-1387.
67. Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J, Gunn RN, Grasby PM, Cowen PJ. Brain $serotonin_{1A}$ receptor binding measured by positron emission tomography with [^{11}C]WAY-100635: effects of depression and antidepressant treatment. *Arch Gen Psychiatry*. 2000;57:174-180.
68. Bhagwagar Z, Rabiner E, Sargent P, Grasby P, Cowen P. Persistent reduction in brain $serotonin_{1A}$ receptor binding in recovered depressed men measured by positron emission tomography with [^{11}C]WAY-100635. *Mol Psychiatry*. 2004;9: 386-392.
69. Neumeister A, Brain E, Nugent A, Carson R, Bonne O, Lucnekbaugh D, Eckelman W, Herschovitch P, Charney D, Drevets W. Reduced serotonin type 1_A receptor binding in panic disorder. *J Neurosci*. 2004;24:589-591.
70. Parsey R, Oquendo M, Simpson N, Ogden R, Van Heertum R, Arango V, Mann J. Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT_{1A} receptor binding potential measured by PET using [C-11]WAY-100635. *Brain Res*. 2002;954:173-182.
71. Cooper SJ. Cholecystokinin modulation of serotonergic control of feeding behavior. *Ann N Y Acad Sci*. 1996;780:213-222.
72. Hajos M, Gartside SE, Varga V, Sharp T. In vivo inhibition of neuronal activity in the rat ventromedial prefrontal cortex by midbrain-raphe nuclei: role of 5-HT_{1A} receptors. *Neuropharmacology*. 2003;45:72-81.
73. Martin-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G, Artigas F. Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. *J Neurosci*. 2001;21: 9856-9866.
74. Bulik CM, Sullivan PF, Joyce PR, Carter FA. Temperament, character, and personality disorder in bulimia nervosa. *J Nerv Ment Dis*. 1995;183:593-598.
75. Klump KL, Bulik CM, Pollice C, Halmi KA, Fichter MM, Berrettini WH, Devlin B, Strober M, Kaplan A, Woodside DB, Treasure J, Shabbout M, Lilenfeld LR, Plotnicov KH, Kaye WH. Temperament and character in women with anorexia nervosa. *J Nerv Ment Dis*. 2000;188:559-567.
76. Kaye W, Bulik C, Thornton L, Barbarich N, Masters K, Fichter M, Halmi K, Kaplan A, Strober M, Woodside DB, Bergen A, Crow S, Mitchell J, Rotondo A, Mauri M, Cassano G, Keel PK, Plotnicov K, Pollice C, Klump K, Lilenfeld LR, Devlin B, Quadflieg R, Berrettini WH. Comorbidity of anxiety disorders with anorexia and bulimia nervosa. *Am J Psychiatry*. 2004;161:2215-2221.
77. Anderluh MB, Tchanturia K, Rabe-Hesketh S, Treasure J. Childhood obsessive-compulsive personality traits in adult women with eating disorders: defining a broader eating disorder phenotype. *Am J Psychiatry*. 2003;160:242-247.
78. Allman J, Hakeem A, Erwin J, Nimchinsky E, Hof P. The anterior cingulate cortex: the evolution of an interface between emotion and cognition. *Ann N Y Acad Sci*. 2001;935:107-117.
79. De Paula Soares V, Zangrossi H Jr. Involvement of 5-HT_{1A} and 5-HT_2 receptors of the dorsal periaqueductal gray in the regulation of the defensive behaviors generated by the elevated T-maze. *Brain Res Bull*. 2004;64:181-188.
80. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. *Brain*. 1995;118:279-306.
81. Takenouchi K, Nishijo H, Uwano T, Tamura R, Takigawa M, Ono T. Emotional and behavioral correlates of the anterior cingulate cortex during associative learning in rats. *Neuroscience*. 1999;93:1271-1287.
82. Bush G, Frazier JA, Rauch SL, Seidman LJ, Whalen PJ, Jenike MA, Rosen BR, Biederman J. Anterior cingulate cortex dysfunction in attention-deficit/hyperactivity disorder revealed by fMRI and the Counting Stroop. *Biol Psychiatry*. 1999;45:1542-1552.
83. Freedman LJ, Insel TR, Smith Y. Subcortical projections of area 25 (subgenual cortex) of the macaque monkey. *J Comp Neurol*. 2000;421:172-188.
84. Osuch EA, Ketter TA, Kimbrell TA, George MS, Benson BE, Willis MW, Herscovitch P, Post RM. Regional cerebral metabolism associated with anxiety symptoms in affective disorder patients. *Biol Psychiatry*. 2000;48:1020-1023.
85. Mayberg HS, Silva JA, Brannan SK, Tekell JL, Mahurin RK, McGinnis S, Jerabek PA. The functional neuroanatomy of the placebo effect. *Am J Psychiatry*. 2002; 159:728-737.
86. Skaf CR, Yamada A, Garrido GE, Buchpiguel CA, Akamine S, Castro CC, Bussato GF. Psychotic symptoms in major depressive disorder are associated with reduced regional cerebral blood flow in the subgenual anterior cingulate cortex: a voxel-based single photon emission computed tomography (SPECT) study. *J Affect Disord*. 2002;68:295-305.
87. de Zwaan M, Aslam Z, Mitchell JE. Research on energy expenditure in individuals with eating disorders: a review. *Int J Eat Disord*. 2002;32:127-134.
88. Charney DS, Deutch A. A functional neuroanatomy of anxiety and fear: implications for the pathophysiology and treatment of anxiety disorders. *Crit Rev Neurobiol*. 1996;10:419-446.
89. Arranz B, Eriksson A, Mellerup E, Plenge P, Marcusson J. Effect of aging in human cortical pre- and postsynaptic serotonin binding sites. *Brain Res*. 1993; 620:163-166.
90. Tauscher J, Pirker W, Willeit M, de Zwaan M, Bailer U, Neumeister A, Asenbaum S, Lennkh C, Praschak-Rieder N, Brücke T, Kasper S. [^{123}I]beta-CIT and single photon emission computed tomography reveal reduced brain serotonin transporter availability in bulimia nervosa. *Biol Psychiatry*. 2001;49:326-332.
91. Palego L, Marazziti D, Rossi A, Giannaccini G, Naccarato AG, Lucacchini A, Cassano GB. Apparent absence of aging and gender effects on serotonin 1A receptors in human neocortex and hippocampus. *Brain Res*. 1997;758:26-32.
92. Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD, Bench CJ, Gunn RN, Cowen P, Grasby PM. A database of [^{11}C]WAY-100635 binding to 5-HT_{1A} receptors in normal male volunteers: normative data and relationship to methodological, demographic, physiological, and behavioral variables. *Neuroimage*. 2002;15:620-632.
93. Slater P, Doyle CA, Deakin JF. Abnormal persistence of cerebellar serotonin-1A receptors in schizophrenia suggests failure to regress in neonates. *J Neural Transm*. 1998;105:305-315.
94. Gunn RN, Sargent PA, Bench CJ, Rabiner EA, Osman S, Pike VW, Hume SP, Grasby PM, Lammertsma AA. Tracer kinetic modeling of the 5-HT1A receptor ligand [carbonyl-11C]WAY- 100635 for PET. *Neuroimage*. 1998;8:426-440.
95. Tauscher J, Bagby RM, Javanmard M, Christensen BK, Kasper S, Kapur S. Inverse relationship between serotonin 5-HT_{1A} receptor binding and anxiety: a [^{11}C]WAY-100635 PET investigation in healthy volunteers. *Am J Psychiatry*. 2001;158:1326-1328.
96. Sullivan PF. Mortality in anorexia nervosa. *Am J Psychiatry*. 1995;152:1073-1074.