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Interaction between serotonin transporter and dopamine D2/D3 receptor radioligand measures is associated with harm avoidant symptoms in anorexia and bulimia nervosa

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Abstract

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Rationale—Individuals with anorexia nervosa (AN) and bulimia nervosa (BN) have alterations of measures of serotonin (5-HT) and dopamine (DA) function, which persist after long-term recovery and are associated with elevated harm avoidance (HA), a measure of anxiety and behavioral inhibition.

Objective—Based on theories that 5-HT is an aversive motivational system that may oppose a DA-related appetitive system, we explored interactions of positron emission tomography (PET) radioligand measures that reflect portions of these systems.

Methods—Twenty-seven individuals recovered (REC) from eating disorders (EDs) (7 AN-BN, 11 AN, 9 BN) and 9 control women (CW) were analyzed for correlations between [^{11}C]McN5652 and [^{11}C]raclopride binding.

Results—There was a positive correlation between [^{11}C]McN5652 binding potential $\text{BP}_{\text{non displacable(ND)}}$ and [^{11}C]raclopride BP_{ND} for the dorsal caudate ($r(27) = .62; p < .001$), antero-ventral striatum ($r(27) = .55, p = .003$), middle caudate ($r(27) = .68; p < .001$), ventral ($r(27) = .64; p < .001$) and dorsal putamen ($r(27) = .42; p = .03$). No significant correlations were found in CW. [^{11}C]raclopride BP_{ND} , but not [^{11}C]McN5652 BP_{ND} , was significantly related to HA in REC EDs. A linear regression analysis showed that the interaction between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} in the dorsal putamen significantly ($b = 140.04; t(22) = 2.21; p = .04$) predicted HA.

Conclusions—This is the first study using PET and the radioligands [^{11}C]McN5652 and [^{11}C]raclopride to show a direct relationship between 5-HT transporter and striatal DA D2/D3 receptor binding in humans, supporting the possibility that 5-HT and DA interactions contribute to HA behaviors in EDs.

Keywords

anorexia nervosa; bulimia nervosa; positron emission tomography; dopamine; serotonin; harm avoidance

1. Introduction

Anorexia nervosa (AN) and bulimia nervosa (BN) are related disorders of unknown etiology that often emerge during adolescence in women (American Psychiatric Association 2000). These disorders are characterized by the relentless pursuit of thinness, obsessive fears of being fat, aberrant eating behaviors, and disturbances of mood and impulse control.

Genetic, pharmacologic, and physiological data (Bergen et al 2005, Friederich et al 2006, Kaye 2008, Kaye et al 1999, Lawrence 2003) suggest that ill and recovered (REC) AN have altered striatal dopamine (DA) function. It remains uncertain whether BN have trait-related DA disturbances because fewer DA studies have been conducted (Jimerson et al 1992, Kaye et al 1990). In terms of positron emission tomography (PET) studies, our group found that REC AN and AN-BN had increased DA D2/D3 binding (e.g. [^{11}C]raclopride binding potential (BP)) in the antero-ventral striatum (AVS) (Frank et al 2005).

REC AN and REC BN have altered (and often different patterns of) 5-HT function (Kaye 2008). For example, studies have shown different degrees of elevated cerebrospinal fluid (CSF) concentrations of 5-Hydroxyindoleacetic Acid (5-HIAA) (Kaye et al 1991, Kaye et al 1998) and altered behavioral responses to 5-HT challenges in these populations (Frank et al 2001, Kaye et al 2003, Smith et al 1999, Ward et al 1998). We have used PET and specific radioligands ([^{11}C]McN5652, [^{11}C]WAY100635 and [^{18}F]altanserin) to assess the BP of the 5-HT transporter (5-HTT) and the 5-HT $_1\text{A}$ and 5-HT $_{2\text{A}}$ receptors. In general, REC ED individuals tend to have reduced 5-HT $_{2\text{A}}$ BP ([^{18}F]altanserin $\text{BP}_{\text{non displacable (ND)}}$) (Bailer

et al 2004, Frank et al 2002, Kaye et al 2001) and elevated 5-HT_{1A} BP ([¹¹C]WAY100635 BP_{plasma free plus protein bound (P)}) (Bailer et al 2011, Bailer et al 2005). Moreover, using PET and [¹¹C]McN5652 our group found REC AN (n = 11) had increased 5-HTT BP ([¹¹C]McN5652 BP_{ND}) in the AVS and the dorsal raphe compared to REC AN-BN (n = 7) (Bailer et al 2007b). However, neither group was statistically different from healthy CW. In contrast, [¹¹C]McN5652 BP_{ND} was normal in REC BN (n = 9), whereas other imaging and peripheral platelet studies have found evidence of reduced 5-HTT in ill and REC BN individuals (Kuikka et al 2001, Steiger et al 2005, Tauscher et al 2001), including a recent study from our group, using [¹¹C]DASB, a more specific radioligand for the 5-HTT (Pichika et al 2012).

It is important to move beyond studying a single monoamine system and address the complex interactions of neural systems (for review see (Tremblay and Blier 2006)) in order to better understand behavior. It has been theorized that 5-HT is the crucial substrate of an aversive motivational system that might oppose a DA-related appetitive system (Cools et al 2008, Daw et al 2002). Specifically, 5-HT has a critical role in the adaptation of animals to aversive events (Bari et al 2010, Deakin and Graeff 1991) and may mediate a negative prediction error signal for future threat and punishment (Boureau and Dayan 2011, Cools et al 2008, Daw et al 2002). While DA has been associated with the expression of an appetitive reward system (Schultz 1997), it is plausible that it works in mutual opponency with a system that signals the prediction of punishment instead of reward (Daw et al 2002, Deakin and Graeff 1991). From another perspective, studies suggest that 5-HT has a role in action choice by controlling the timescale of delayed rewards through differential effects on ventral and dorsal striatal circuits (McClure et al 2004, Schweighofer et al 2007).

We are interested in exploring how complex monoamine interactions may be related to diagnostic category (e.g., AN vs. BN), impulse control, anxiety, and other behaviors that may cut across diagnostic boundaries. In terms of the latter, our PET imaging studies show striking and consistent correlations between the BP of both 5-HT_{1A} and 5-HT_{2A}, DA D2/D3 receptors, and HA among all subgroups of ED individuals. HA is associated with 5-HT_{1A} BP_P in REC AN (Bailer et al 2005) and REC BN (Bailer et al 2011), 5-HT_{2A} BP_{ND} in REC AN-BN (Bailer et al 2004) and ill AN/AN-BN (Bailer et al 2007a), and DA D2/D3 BP_{ND} in REC AN/AN-BN (Frank et al 2005). In addition to being a premorbid influencing factor (Anderluh et al 2003, Lilenfeld et al 2006, Stice 2002), elevated HA is commonly found among all ill ED subtypes [see review by Cassin et al. (Cassin and von Ranson 2005)] and persists after recovery from AN and BN (Wagner et al 2006). HA is a multifaceted temperament trait (Cloninger et al 1994a) that contains elements of anxiety, inhibition, and inflexibility; it reflects the construct of ‘behavioral inhibition’.

The main purpose of this study was to use our previously published PET data to explore whether 5-HT and DA interactions occur in REC ED subjects, beyond diagnostic boundaries, and whether such interactions were related to HA or to other behaviors. We have not found significant relationships between [¹¹C]raclopride BP_{ND} or [¹¹C]WAY100635 BP_P or [¹⁸F]altanserin BP_{ND} (unpublished data). The only significant relationships were between [¹¹C]McN5652 BP_{ND} and [¹¹C]raclopride BP_{ND}, indicating an interaction between 5-HTT and DA D2/D3 binding, which is the focus of this paper. The [¹¹C]McN5652 BP_{ND} data has been published elsewhere (Bailer et al 2007b). The current paper presents new [¹¹C]raclopride BP_{ND} data on REC ED subjects and CW in order to explore these interaction findings.

2. Methods

2.1. Subjects

Forty-five women who were REC from EDs were studied with PET imaging and the radioligand [^{11}C]raclopride. The sample consisted of 17 REC AN [4 part of (Frank et al 2005)], 14 REC AN-BN [6 part of (Frank et al 2005)], 14 REC BN, and 21 CW [12 part of (Frank et al 2005)]. Out of this initial sample, a smaller sample of 27 REC EDs (11 REC AN, 7 REC AN-BN, 9 REC BN) and 9 CW were analyzed for correlations between [^{11}C]McN5652 and [^{11}C]raclopride binding, as these subjects completed both PET studies. Data on some subjects (of this smaller sample) regarding [^{11}C]McN5652 binding (11 REC AN, 7 REC AN-BN, 9 REC BN, 9 CW) and [^{11}C]raclopride binding (4 REC AN, 6 REC AN-BN, 9 CW) were recently published (Bailer et al 2007b, Frank et al 2005). We are presenting all of the [^{11}C]raclopride data because it would be misleading to only present the [^{11}C]raclopride data on the group who also had [^{11}C]McN5652 data.

Subjects were recruited as previously described (Wagner et al 2006). Women between the ages of 18 and 45 years who had previously met criteria for AN or BN as defined in the 4th ed. of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association 2000) were recruited. They were previously treated in the ED treatment program at Western Psychiatric Institute and Clinic (University of Pittsburgh Medical Center, Pittsburgh, PA) or were recruited nationally through advertisements. All individuals underwent 4 levels of screening: (a) a brief telephone screening; (b) an intensive screening process to assess psychiatric history, lifetime weight, binge eating and methods of weight loss/control, and menstrual cycle history as well as their eating pattern for the past 12 months; (c) a comprehensive assessment using structured and semistructured psychiatric interviews conducted by telephone or in person; and (d) a face-to-face interview and physical examination with a psychiatrist. In order to be considered REC, subjects had to: 1) maintain a weight above 90% average body weight (MetropolitanLifeInsuranceCompany 1959), 2) have regular menstrual cycles; and 3) not have binged, purged, or engaged in significant restrictive eating patterns, or exercised excessively; and 4) not used psychoactive medications such as antidepressants for at least 1 year prior to the study. Also, they must not have met criteria for current alcohol or drug abuse/dependence.

Because of the small group size, we combined all individuals who met criteria for binge/purge type AN and individuals who met lifetime criteria for both AN and BN during the course of their illness (referred to as REC AN-BN) after determining by a Mann-Whitney test that these groups did not differ on any of the dependent measures (data not shown). CW were recruited through local advertisements. They had no history of an ED or any psychiatric disorder and no serious medical or neurologic illness. They had normal menstrual cycles and had been within normal weight range since menarche (> 90% of average body weight). This study was conducted according to the institutional review board regulations of the University of Pittsburgh, and all subjects gave written informed consent. During the screening process, current psychopathology was assessed, both in REC ED and CW, with a comprehensive battery of standardized instruments [see Wagner *et al.* (Wagner et al 2006)], designed to diagnose Axis I and II disorders and to assess symptoms typical in individuals with EDs. The battery included the Temperament and Character Inventory (Cloninger et al 1994b) for assessment of HA, novelty seeking (NS), reward dependence (RD and persistence (P)). Trained doctoral-level psychologists with experience in ED administered the clinical interviews.

2.2. Image acquisition

All subjects were scanned on the same ECAT HR+ PET scanner (CTI PET systems, Knoxville, TN), located at the University of Pittsburgh PET center, in three-dimensional (3D) imaging mode during the first 10 days of the follicular phase of the menstrual cycle. All subjects also underwent magnetic resonance (MR) imaging before the PET scan on a Signa 1.5 Tesla scanner (GE Medical Systems, Milwaukee, WI) using a standard head coil. A volumetric spoiled gradient recalled (SPGR) sequence (TE = 5, TR = 25, flip angle = 40°, NEX = 1; field of view = 24 cm, image matrix = 256 = 192 pixels) acquired in the coronal plane was used for MR-PET image registration (to guide region of interest (ROI) placement) and for partial volume correction of the PET data. Fast spin-echo T2 and proton density weighted images were also routinely acquired to exclude significant neuropathology. Pixels that corresponded to scalp and calvarium were removed from the SPGR MR images (Sandor and Leahy 1997). The MR-PET image alignment was performed using an automated method for centering (Minoshima et al 1992) and alignment and reslicing (Woods et al 1993). The PET image data were aligned to the SPGR MR data to yield registration parameters. The MR data were then resliced using the inverse transformation of the PET-to-MR alignment to match PET image space. The data were visually inspected for subject motion and inter-frame motion was corrected by applying a more extensive registration procedure on a frame-by-frame basis.

Immediately following slow bolus intravenous injection of 13.8 ± 1.8 mCi [^{11}C]McN5652, dynamic emission scanning with arterial blood sampling (input function) was performed over 90 minutes (Bailer et al 2007b, Lopresti et al 2001). Additionally, after slow bolus intravenous injection of high specific activity 10.4 ± 0.5 mCi [^{11}C]raclopride, dynamic emission scanning with arterial blood sampling (input function) was performed over 60 minutes, as described previously (Drevets et al 2001, Frank et al 2005). Whenever possible, the two PET scans were done on the same day. Only 4 REC EDs (2 REC AN, 2 REC BN) and 3 CW had the scans on different days, ranging from 1 day to a maximum of 12 weeks interval. However, test-retest measures are high for both radioligands (Hietala et al 1999, Kent et al 2002, Schlosser et al 1998, Szabo et al 2002, Uchida et al 2009, Volkow et al 1993) and indicate that [^{11}C]McN5652 and [^{11}C]raclopride binding is stable when measured over weeks to months.

2.3. PET data processing

ROIs were hand drawn on the coregistered MR images, blind to subjects' diagnosis and applied to the dynamic PET data to generate time-activity curves, as described by Drevets et al. (Drevets et al 2001): AVS, dorsal and middle caudate, dorsal and ventral putamen, and cerebellum (as a reference region) (Bailer et al 2005, Drevets et al 2001, Frank et al 2005). In addition to these ROIs, the dorsal raphe nucleus was chosen. For the kinetic analyses, regional [^{11}C]McN5652 volume of distribution (V_T) was determined using a 2-compartment, 3-parameter tracer kinetic model (Innis et al 2007). Specific 5-HTT binding was assessed using the BP_{ND} measure which is based upon the ratio of each ROI V_T to the cerebellar volume of distribution (V_{ND}) [(V_T/V_{ND})], where $BP_{ND} = (V_T - V_{ND})/V_{ND}$ (Parsey et al 2000). Due to the fact that arterial blood sampling was not available in all subjects, the Reference Tissue Model (Lammertsma and Hume 1996) was applied for the imaging data analysis of [^{11}C]raclopride, which has been shown to be an appropriate and robust model for quantifying [^{11}C]raclopride (Drevets et al 2001, Frank et al 2005). Here, the [^{11}C]raclopride BP_{ND} was derived from the kinetic rate constant k_3/k_4 , which is equivalent to $(V_T - V_{ND})/V_{ND}$ (Innis et al 2007). An MR-based partial volume (PV) effect 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, Meltzer et al 1999).

2.4. Statistical Analysis

Correlations were examined with Pearson correlation coefficients, demographics and assessments between groups were compared with a one-way analysis of variance (ANOVA). We used Troendle's method (Troendle 1995) to adjust for the multiple correlations between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND}. This resampling method for multiple hypothesis testing is designed to deal with the fact that multiple test statistics computed over a single dataset will tend to be dependent. It determines the appropriate correction directly from the data, as follows. First, the raw test statistics are computed and the hypothesis corresponding to the largest of these is noted. Then, resampling is applied to the dataset to determine the null distribution of the maximum test statistic. The largest observed test statistic is compared to this null distribution and the corresponding null hypothesis is rejected if the p-value is below the desired family-wise error rate (set to 0.05). If this hypothesis is not rejected, the procedure stops and no null hypotheses are rejected. On the other hand, if it is rejected, that hypothesis is removed from the list of those to be tested and the procedure starts again with the new list, repeating until we fail to reject the remaining null hypotheses. [^{11}C]raclopride BP_{ND} was regressed on age and t-tests of age-adjusted values within the General Linear model in the statistical computing environment R version 2.9.0 (2009-04-17) (RDevelopmentCoreTeam 2011) were used to compare [^{11}C]raclopride BP_{ND} between CW and REC AN, REC BN, REC AN-BN, respectively. Additional linear regression analyses that included an interaction term were used to assess the extent to which the interaction between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} predicted HA. Prior to performing the regression analyses, assessment of possible outliers showed one individual who displayed a [^{11}C]McN5652 BP_{ND} value that was more than 3 standard deviations above the mean; this individual was removed from the analysis.

3. Results

3.1. Demographics and assessments

Demographics and assessments, shown in Table 1, are displayed for the entire sample ($n = 66$) of REC ED and CW for the [^{11}C]raclopride BP_{ND}, and for the smaller sample ($n = 36$) for the correlational analysis between [^{11}C]raclopride BP_{ND} and [^{11}C]McN5652 BP_{ND}. Neither a lifetime history of major depressive disorder nor the number of previous depressive episodes were related to [^{11}C]McN5652 BP_{ND} ($n=16$; 5 REC AN, 4 REC AN-BN, and 7 REC BN (Bailer et al 2007b)) or to [^{11}C]raclopride BP_{ND} ($n = 34$; 10 REC AN, 11 REC AN-BN; 13 REC BN).

3.2. [^{11}C]raclopride BP_{ND}

[^{11}C]raclopride BP_{ND} in the AVS differed significantly between CW and REC AN women ($t [36] = 2.148, p = 0.036$) (Figure 1) after adjusting for the effects of age. However, this significance would not survive a correction for multiple comparisons. Otherwise, there were no significant differences between REC ED groups and CW for the AVS or any of the other regions (Table 2).

3.3. Relationship of [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND}

In the sample of 27 REC EDs there was a positive correlation between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} for the dorsal caudate ($r(27) = .62; p < .001$) (Figure 2A), AVS ($r(27) = .55, p = .003$) (Figure 2B), middle caudate ($r(27) = .68; p < .001$), ventral putamen ($r(27) = .64; p < .001$) and dorsal putamen ($r(27) = .42; p = .03$). The correlation between [^{11}C]McN5652 BP_{ND} in the dorsal raphe and [^{11}C]raclopride BP_{ND} in the AVS was trending in the positive direction ($r(27) = .35; p = .07$). After correction using Troendle's method (Troendle 1995), the correlations within the dorsal caudate ($p_{\text{adjusted}} = .$

026), AVS ($p_{\text{adjusted}} = .047$), middle caudate ($p_{\text{adjusted}} = .004$), and ventral putamen ($p_{\text{adjusted}} = .010$) remained significant. No significant correlations were found within individual ED subgroups (REC AN, REC AN-BN, REC BN) between [^{11}C]raclopride BP_{ND} and [^{11}C]McN5652 BP_{ND} . In the small sample of only 9 CW who completed both [^{11}C]McN5652 and [^{11}C]raclopride PET studies, no significant correlations were found between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} .

When using PV-uncorrected values for both [^{11}C]raclopride and [^{11}C]McN5652 BP_{ND} in the sample of 27 REC EDs there was a positive correlation between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} for the dorsal caudate ($r(27) = .62$; $p = .001$), AVS ($r(27) = .56$, $p = .002$), middle caudate ($r(27) = .66$; $p < .001$), ventral putamen ($r(27) = .60$; $p = .001$) and dorsal putamen ($r(27) = .42$; $p = .03$). This is quite similar to what we found with the PV corrected values (see above). In addition, there were significant correlations between [^{11}C]McN5652 BP_{ND} in the dorsal raphe and [^{11}C]raclopride BP_{ND} in the AVS ($r(27) = .52$; $p = .006$), dorsal caudate ($r(27) = .40$; $p = .04$), dorsal putamen ($r(27) = .39$; $p = .05$), middle caudate ($r(27) = .58$; $p = .002$) and ventral putamen ($r(27) = .60$; $p = .001$). No significant correlations were found within each individual ED subgroup (REC AN, REC AN-BN, REC BN) between [^{11}C]raclopride BP_{ND} and [^{11}C]McN5652 BP_{ND} . In the small sample of only 9 CW who completed both [^{11}C]McN5652 and [^{11}C]raclopride PET studies, no significant correlations were found between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} , when using PV-uncorrected values.

3.4. Relationship of [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} and HA

REC EDs showed positive correlations between [^{11}C]raclopride BP_{ND} and HA in the dorsal caudate, ($r(27) = .48$; $p = .01$) (Figure 3A) and dorsal putamen ($r(27) = .42$; $p = .03$) (Figure 3B). There was no significant correlation between [^{11}C]McN5652 BP_{ND} and HA in any of the ROIs; however, linear regression analysis revealed that the interaction between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} in the dorsal putamen was significantly associated with HA ($b = 140.04$; $t(22) = 2.21$; $p = 0.04$) (Figure 4A). In the dorsal caudate, the same interaction approached significance ($b = -60.43$; $t(22) = -1.725$; $p = 0.10$) (Figure 4B). The AICs for each of the three models in the dorsal putamen (association between [^{11}C]raclopride BP_{ND} and HA, [^{11}C]McN5652 BP_{ND} and HA, the interaction term and HA) were 258.716, 258.282 and 256.327, respectively. The model with the interaction term had the lowest AIC and was selected as most predictive using this approach. More precisely, HA was regressed on [^{11}C]McN5652 BP_{ND} , and [^{11}C]raclopride BP_{ND} , in the dorsal putamen, with and without their interaction. When the interaction term was not included, neither BP_{ND} were significantly predictive of HA ($b\text{-McN} = 11.902$, $se = 10.200$, $p = 0.252$; $b\text{-rac} = 4.755$, $se = 4.878$, $p = 0.337$). However, when the interaction term was included, both the interaction and the [^{11}C]McN5652 BP_{ND} term were statistically significant ($b\text{-McN} = -219.92$, $se = 107.38$, $p = 0.0488$; $b\text{-rac} = -42.73$, $se = 22.39$, $p = 0.0653$; $b\text{-interaction} = 73.72$, $se = 34.01$, $p = 0.0377$). No significant correlations were found between HA and [^{11}C]McN5652 BP_{ND} or [^{11}C]raclopride BP_{ND} in CW. Other behaviors, body mass index, or length of recovery were not related to either [^{11}C]McN5652 BP_{ND} or [^{11}C]raclopride BP_{ND} or HA, NS, RD or P.

4. Discussion

To our knowledge, this is the first imaging study to show a relationship between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} in humans. This study showed that [^{11}C]raclopride BP_{ND} , but not [^{11}C]McN5652 BP_{ND} , was significantly related to HA in REC ED. A linear regression analysis revealed that the interaction between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} in the dorsal putamen significantly predicted HA.

The physiology underlying 5-HT, DA, and HA relationships is not well understood. HA, a continuous measure of inhibition and anxiety, was thought to reflect central serotonergic turnover (Cloninger 1988, Cloninger 1986, Cloninger et al 1993). There is extensive literature associating 5-HT system activity with fundamental aspects of behavioral inhibition (Geyer 1996), e.g., reduced CSF 5-HIAA levels are associated with increased impulsivity and aggression in humans and non-human primates, whereas increased CSF 5-HIAA levels are related to behavioral inhibition (Fairbanks et al 2001, Schweighofer et al 2007, Westergaard et al 2003). In fact, there is considerable evidence that 5-HT function is inhibitory of appetite and plays a role in anxious and obsessive behaviors, as well as in depression (Blundell 1984, Cloninger 1987, Higley and Linnoila 1997, Leibowitz and Shor-Posner 1986, Lucki 1998, Mann 1999, Soubrie 1986).

As noted above, our previous PET imaging studies have shown consistent correlations between HA and the BP for 5-HT_{1A}, or 5-HT_{2A}, or DA D2/D3 receptors, in all subgroups of ED individuals. In specific, HA was associated with mesial temporal 5-HT_{1A} BP_P in REC AN (Bailer et al 2005), lateral and medial orbital frontal as well as parietal 5-HT_{1A} BP_P in REC BN (Bailer et al 2011), subgenual cingulate and temporal 5-HT_{2A} BP_{ND} in REC AN-BN (Bailer et al 2004), subgenual cingulate, lateral and medial orbital frontal and parietal 5-HT_{2A} BP_{ND} in ill AN/AN-BN (Bailer et al 2007a). In addition, HA has also been associated with caudate DA activity in Parkinson's disease (Kaasinen et al 2001), and a relationship between striatal DA activity and trait anxiety has been reported in healthy controls (Laakso et al 2003). Moreover, dorsal caudate and dorsal putamen DA D2/D3 BP_{ND} was positively associated with HA in REC AN/AN-BN (Frank et al 2005).

Interestingly, we did not find an interaction between 5-HT_{1A} and/or 5-HT_{2A} and DA D2/D3 binding with HA (data not shown). Nor did we find a direct relationship between 5-HTT binding and HA. Rather, 5-HTT binding was associated with HA, but only in relationship to 5-HTT and DA interactions, raising the possibility that 5-HTT may have a modulatory role on DA activity, with the latter being associated with HA. In support of this idea, agents that are relatively 5-HT specific, such as psilocybin (Vollenweider et al 1999), fenfluramine (Smith et al 1997), and citalopram (Tiihonen et al 1996) alter [¹¹C]raclopride binding. It is important to note that 5-HT and DA neural systems are complex. For example, the 5-HT system has 14 or more receptors and many other components that modulate metabolism, firing rate, neuronal cascades, etc. Thus it is possible that interactions between 5-HT and DA could involve elements not measured in this study, which might contribute to the paradoxical finding of interactions between 5-HTT and DA D2/D3 that showed a positive association with HA in the dorsal putamen but a negative one in the dorsal caudate. A review of the literature did not find any studies that have investigated interactions between 5-HTT and DA D2/D3 receptors. However, 5-HT_{2C} receptors tonically inhibit mesencephalic DA neurons (De Deurwaerdere et al 2004). Administration of the selective 5-HT_{2C} agonist RO 60-0175 decreases basal DA release in the nucleus accumbens and decreases basal firing rate of DA neurons in the ventral tegmental area (VTA) (Di Matteo et al 2000). The 5-HT_{2C/2B} antagonist SB 206553 increases basal DA release in the nucleus accumbens and striatum and increases basal firing rate of DA neurons in the VTA and substantia nigra pars compacta (Di Giovanni et al 1999).

One limitation of PET radioligand studies is that they provide a relatively narrow window into understanding complex neurotransmission function. This is an especially important consideration in our attempts to study 5-HT, as a radiotracer of equivalent value as [¹¹C]raclopride has been to dopamine has not yet been identified (for review see Paterson et al, 2010 (Paterson et al 2010)). For example, [¹¹C]McN5652 BP_{ND} presumably reflects 5-HTT density and/or affinity because [¹¹C]McN5652 is not displaced from 5-HTT sites by physiologically relevant 5-HT concentrations (Hummerich et al 2006, Meyer 2007).

Therefore, [^{11}C]McN5652 BP_{ND} appears to remain relatively unaffected by endogenous 5-HT (Hummerich et al 2006). One model, as suggested by Meyer et al. (Meyer 2007), proposes a clearance effect of 5-HTT, with less functioning 5-HTT associated with greater extracellular 5-HT. Theoretically, individuals with greater [^{11}C]McN5652 BP_{ND} have reduced extracellular 5-HT. This is consistent with our studies of CSF 5-HIAA in REC AN and BN (Kaye et al 1991, Kaye et al 1998), where REC AN have relatively lower concentrations compared to REC BN, which corresponds with higher purported 5-HTT binding in REC AN compared to REC BN (Bailer et al 2007b). One possibility, although conjectural, is that those individuals with elevated [^{11}C]McN5652 BP_{ND} have reduced extracellular 5-HT. In turn, there would be diminished 5-HT stimulation of 5-HT_{2C} post-synaptic receptors. As described, studies in animals show that 5-HT_{2C} receptors tonically inhibit mesencephalic DA neurons (De Deurwaerdere et al 2004). Less 5-HT_{2C} inhibition might result in less inhibition of DA neurons. Sustained DA activation has been shown to be related to uncertainty (Fiorillo et al 2003), which could be consistent with behavioral measures of elevated anxiety and inhibition - and HA - in some individuals with ED.

It has been shown in animals (Morris et al 1999) and humans (Volkow et al 1996, Volkow et al 2000) that [^{11}C]raclopride BP_{ND} diminishes with age. When corrected for age, as done in other [^{11}C]raclopride PET studies (Reeves et al 2005, Reeves et al 2007), this study found that REC AN (but not REC AN-BN or REC BN) had increased AVS [^{11}C]raclopride BP_{ND} when compared to CW (uncorrected for multiple comparisons). This finding corresponds with our previous report of increased [^{11}C]raclopride BP_{ND} in REC AN/AN-BN, where AVS [^{11}C]raclopride BP_{ND} in REC AN tended to be higher compared to REC AN-BN (Frank et al 2005). [^{11}C]raclopride BP_{ND} is influenced by endogenous DA; therefore, the BP_{ND} is interpreted as a measure of receptor availability, rather than absolute receptor density [see (Drevets et al 2001, Graff-Guerrero et al 2008, Zald et al 2010)]. Thus, elevated AVS [^{11}C]raclopride BP_{ND} could indicate either an elevation of the density and/or affinity of the D2/D3 receptors or a reduction in intrasynaptic DA concentrations. The latter would be consistent with our previous findings of reduced CSF homovanillic acid (HVA) concentrations in REC AN and normal CSF HVA levels for REC AN-BN and BN subjects (Kaye et al 1999). It is important to note that DA D2/D3 receptors are just one small part of a complex DA system that involves the interaction of a number of DA receptors and other molecules. Moreover, DA D2/D3 receptors show opposite roles in subregions of the nucleus accumbens which may confound interpretation of findings (Besson et al 2010). In addition, there are differences within striatal regions in terms of the relative balance of D2 and D3 receptors (Gurevich and Joyce 1999), expression of the DA transporter (Martinez et al 2003), and DA D1 receptor density (Muly et al 2010).

Limitations

The main focus of this paper was the interaction of [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} and its relationship to behavior in REC ED. First, the sample of CW that had [^{11}C]McN5652 BP_{ND} studies was about half of the entire sample that had [^{11}C]raclopride data available, making it too small and underpowered to draw conclusions about relationships that might occur in healthy populations. Secondly, the interactions between 5-HTT, DA D2/D3 and HA would not survive a correction for multiple comparisons. Lastly, small sample sizes and insufficient power to detect differences between groups also applied to the ED subgroup samples (7 REC AN-BN, 11 REC AN, 9 REC BN). Therefore, studies with large enough samples to detect subgroup differences will need to be completed in the future.

It is unclear whether the 5-HTT is itself abnormal in ED subjects or whether disturbed 5-HTT binding may be secondary to other 5-HT disturbances (Lage et al 2010). If so, it is possible that [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} may serve as useful

“markers” of 5-HT and DA neural activity. In this regard, it is possible that [^{11}C]raclopride BP_{ND} and [^{11}C]McN5652 BP_{ND} interactions or [^{11}C]raclopride BP_{ND} and HA relationships reflect a balance between 5-HT functional activity (aversive or inhibitory) and DA functional activity (reward or motivation) (Cools et al 2008, Daw et al 2002). However aspects suggesting a competition or a collaboration or neither between serotonin and dopamine function are manifold making it difficult to draw conclusions regarding the nature of their relationship (see Boureau & Dayan 2011 (Boureau and Dayan 2011) for review). Finally, DA, and 5-HT systems have reciprocal interactions making it virtually impossible to act on a specific neuronal element without a cascade effect on the other systems (Tremblay and Blier 2006).

Conclusion

In summary, positive correlations between PET radioligand measures reflecting binding of the 5-HTT and DA D2/D3 receptors in the AVS and dorsal caudate were found. DA D2/D3 receptor binding in the dorsal caudate, as well as interactions between DA D2/D3 receptor and 5-HTT binding, were related to HA scores supporting the possibility that 5-HT and DA interactions contribute to HA behaviors in EDs.

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Figure 1. Comparison of [¹¹C]raclopride BP_{ND} in the antero-ventral striatum between groups; CW, control women; REC, recovered; AN, anorexia nervosa, restricting type AN; AN-BN, bulimic type AN; BN, bulimia nervosa

Figure 2.

Correlation of $[^{11}\text{C}]\text{McN5652 BPND}$ and $[^{11}\text{C}]\text{raclopride BPND}$ in the dorsal caudate (Panel A) and antero-ventral striatum (AVS) (Panel B) in the REC ED sample

Figure 3.

REC ED showed positive correlations between $[^{11}\text{C}]\text{raclopride BP}_{\text{ND}}$ and Harm Avoidance in the dorsal caudate ($r(27) = .48$; $p = .01$) (Panel A) and dorsal putamen ($r(27) = .42$; $p = .03$) (Panel B)

Figure 4.

For each region, scatterplots of [^{11}C]McN5652 BP_{ND} (y-axis) versus Harm Avoidance (x-axis), with each individual coded with a symbol based on a median split of [^{11}C]raclopride BP_{ND}, depict the relationship between these three continuous variables.

Panel A.

In the dorsal putamen, women with [^{11}C]raclopride BP_{ND} values below the median show a negative relationship between [^{11}C]McN5652 BP_{ND} and Harm Avoidance, while those with [^{11}C]raclopride BP_{ND} values above the median show a positive relationship between [^{11}C]McN5652 BP_{ND} and Harm Avoidance.

Panel B.

In the dorsal caudate, women with [^{11}C]raclopride BP_{ND} values below the median show a slight positive relationship between [^{11}C]McN5652 BP_{ND} and Harm Avoidance, while those with [^{11}C]raclopride BP_{ND} values above the median show a negative relationship between [^{11}C]McN5652 BP_{ND} and Harm Avoidance.

Table 1

Demographic values and assessments between groups

Entire sample for [¹¹ C]raclopride BP _{ND}							
	CW (n=21) ¹	REC AN (n=17) ²	REC AN-BN (n=14) ³	REC BN (n=14) ⁴	F [df]	Sig.	Group diff.
Age (years)	27.7 (7.1)	25.6 (6.4)	28.1 (7.2)	26.3 (7.2)	0.46 [3,62]	0.71	
Current BMI (kg/m ²)	22.51 (2.26)	20.69 (2.00)	22.85 (2.82)	22.81 (2.34)	3.17 [3,62]	0.04	2 < 3 *
Harm Avoidance	10.30 (5.27) [#]	18.69 (7.36) [#]	16.43 (8.05)	19.35 (7.61)	6.27 [3,60]	0.001	1 < 2, 4
Sample for relationships between [¹¹ C]raclopride BP _{ND} and [¹¹ C]McN5652 BP _{ND}							
	CW (n=9) ¹	REC AN (n=11) ²	REC AN-BN (n=7) ³	REC BN (n=9) ⁴	F [df]	Sig.	Group diff.
Age (years)	27.0 (6.4)	24.7 (5.5)	23.7 (4.2)	24.4 (5.3)	0.60 [3,32]	0.62	
Current BMI (kg/m ²)	22.45 (2.04)	20.74 (2.44)	21.75 (2.27)	23.24 (2.76)	3.16 [3,32]	0.04	2 < 4
Age of onset (years of age)	---	15.9 (3.2)	15.8 (1.9) [#]	17.4 (4.7) [#]	0.49 [2,22]	0.62	
Length of recovery (months)	---	43.4 (49.5)	12.0 (13.6) [#]	17.1 (10.2) [#]	2.13 [2,22]	0.14	
Harm Avoidance	7.78 (3.96)	16.18 (7.97)	12.86 (10.78)	19.88 (6.28)	4.31 [3,32]	0.01	1 < 4
Novelty Seeking	21.00 (5.27)	17.00 (7.51)	21.71 (8.50)	20.32 (8.28)	0.77 [3,32]	0.52	
Reward Dependence	19.22 (2.95)	16.73 (3.90)	18.80 (2.36)	19.38 (2.74)	1.58 [3,32]	0.21	
Persistence	4.56 (1.42)	6.91 (1.51)	6.86 (1.35)	6.00 (1.73)	4.72 [3,32]	0.01	1 < 2, 3

Sig., Significance; CW, control women; REC, recovered; AN, anorexia nervosa, restricting type AN; AN-BN, bulimic type AN; BN, bulimia nervosa;

* at a trend level (p = .07);

[#] one missing value

Table 2

Regional [¹¹C]raclopride BP_{ND} between groups

ROI	CW (n=21) ¹		REC AN (n=17) ²		REC AN-BN (n=14) ³		REC BN (n=14) ⁴		2 vs 1		3 vs 1		4 vs 1	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	T [df]	P	T [df]	P	T [df]	P
antero-ventral striatum	2.120	0.436	2.404	0.268	2.147	0.262	2.299	0.473	2.148 [36]	0.036	0.246 [33]	0.807	1.281 [33]	0.205
dorsal caudate	2.622	0.365	2.541	0.345	2.528	0.367	2.729	0.351	-0.711 [36]	0.480	-0.750 [33]	0.456	0.845 [33]	0.402
dorsal putamen	2.921	0.579	2.965	0.328	2.838	0.253	3.087	0.338	0.168 [36]	0.867	-0.553 [33]	0.582	1.059 [33]	0.294
ventral putamen	2.841	0.506	3.018	0.288	2.867	0.242	3.086	0.431	1.194 [36]	0.237	0.230 [33]	0.819	1.698 [33]	0.095
middle caudate	2.516	0.293	2.617	0.342	2.450	0.224	2.658	0.391	0.668 [36]	0.506	-0.576 [33]	0.567	1.154 [33]	0.253

ROI, region of interest; CW, control women; REC, recovered; AN, anorexia nervosa, restricting type AN; AN-BN, bulimic type AN; BN, bulimia nervosa