UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN

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1. PROJECT TITLE

Monoamine Contributions to Neurocircuitry in Eating Disorders

2. PRINCIPAL INVESTIGATOR

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4. ESTIMATED DURATION OF THE STUDY

5 years

5. LAY LANGUAGE SUMMARY OR SYNOPSIS (no more than one paragraph)

Evidence suggests that the regulation of the neurotransmitters serotonin and dopamine is altered in the brain of people with anorexia nervosa (AN) and bulimia nervosa (BN). This study will use brain imaging technologies to measure several neurotransmitters (serotonin and dopamine) that contribute to our abilities to respond to reward or inhibit our impulses, and which are known to be altered in the brain of people with anorexia nervosa (AN) and bulimia nervosa (BN). Because palatable food stimulates dopamine secretion, we propose to use a challenge with brain imaging that will stimulate dopamine release which we hypothesize will generate anxiety rather than pleasure in AN, and will help explain why AN restrict eating in order to reduce anxiety. This application will help to understand the unique and puzzling symptoms of eating disorders and contribute to finding better methods for identifying effective treatments for these often relapsing and sometimes chronic disorders.

6. SPECIFIC AIMS

How are individuals with restricting-type AN able to starve themselves and become emaciated while most people have difficulty losing a few pounds? Furthermore, why do individuals with BN tend to periodically overeat? Considerable data support our overarching hypothesis: Serotonin (5-HT) and dopamine (DA) functional activity, through actions on reward and inhibitory neural pathways, contribute to disturbed appetitive behaviors and extremes of self-control in AN and BN. In order to avoid the confounding effects of malnutrition and weight loss (and because our data suggest that recovered (REC) individuals have persistent trait-related alterations of DA and 5-HT function,[11, 12, 14, 60] we will study, over the course of 5 years, 25 REC AN, 25 REC AN-BN, 25 REC BN, and 25 age and body mass index (BMI) matched healthy control women (CW) who are 18 to 45 years old. We will use PET imaging with specific radioligands for the DA D2/D3 receptors ([^{11}C]raclopride) and 5-HTT ([^{11}C]DASB).

It is well known that 5-HT functional activity inhibits feeding.[23, 110] Considerable data[90] suggest that AN and BN have different disturbances of 5-HT neural circuitry, which contributes to both restricted eating and overeating. **In AIM 1**, our data[14, 138] support the prediction that REC AN will have elevated, and REC BN will have diminished 5-HTT binding [e.g., [^{11}C]DASB binding potential ($BP_{Nondisplaceable\ tissue\ uptake\ (ND)}$)] in midbrain and striatal regions) contributing to inhibition of appetite and other rewards in AN, and decreased self-regulatory control in BN. We predict REC AN-BN will have 5-HTT binding values that fall in-between REC AN and REC BN.

DA circuits play a key role in optimal response to stimuli. Our data support the possibility that AN and BN may have similar dysregulation of anterior ventral striatum (AVS) system function, making them vulnerable to dysfunctional modulation of the rewarding aspects of appetitive behaviors (and modulation of emotionality).
In **Aim 2A,** we propose to use PET and [^{11}C]raclopride binding to confirm several hypotheses generated by our preliminary data regarding striatal DA D2/D3 receptors[9, 60]. First, that REC AN, and perhaps REC BN, have elevated anterior ventral striatum (AVS) [^{11}C]raclopride (DA D2/D3 receptor) BP_{ND} compared to CW. Second, that dorsal caudate (DC) [^{11}C]raclopride BP_{ND} in REC eating disorders (EDs) is associated with baseline harm avoidance (HA), a measure of inhibition and anxiety.
In **AIM 2B,** we will assess endogenous striatal DA release in order to better understand altered striatal [^{11}C]raclopride binding, and (in **AIM 2C**) to replicate and extend pilot data[10] that show that endogenous DA release in the dorsal caudate (DC) provokes inhibitory and anxiety symptoms in AN and BN. In brief, because [^{11}C]raclopride binding is influenced by endogenous DA, elevated [^{11}C]raclopride binding could indicate either an elevation of the density and/or affinity of the DA D2/D3 receptors or a reduction of intrasynaptic DA concentrations. It has been shown in non-human primate studies that the decrease in the BP_{ND} of benzamide radiotracers such as [^{11}C]raclopride following amphetamine (AMPH)-induced DA release is a reliable measure of endogenous DA transmission[29, 108] in striatal subdivisions (limbic, associative and sensorimotor striatum).[121, 122] AMPH administration results in an increase in extracellular DA and a consequent reduction in [^{11}C]raclopride binding. The change (Δ) in BP_{ND} (the difference between the [^{11}C]raclopride BP_{ND} at baseline and post-AMPH treatment normalized to the baseline BP_{ND} and expressed as a percentage) provides a non-invasive measure of changes in DA concentration in the human brain. We will use the PET/double [^{11}C]raclopride/AMPH paradigm described above to assess striatal endogenous DA release. Several recent, small scale studies using this technology[10, 31] and a previous study[96] of cerebral spinal fluid (CSF) homovanillic acid (HVA), a DA metabolite, support the likelihood that REC AN and BN have alterations of endogenous striatal DA release. In AIM 2B we will assess endogenous DA release to help interpret alterations in DA functional activity as well as D2/D3 receptors in limbic, associative, and sensorimotor striatal regions._As summarized in **Table 1** we predict that endogenous DA release will be selectively diminished in limbic striatal regions in REC AN and in associative and sensorimotor regions in REC BN. These data argue that AN have diminished ability to direct "motivated" responses whereas BN may have hypoactive dorsal associative inhibitory pathways. Several lines of evidence support our hypothesis that alterations of food-induced DA secretion, perhaps acting on DA D2 receptors, contribute to pathologic eating. In **AIM 2C** we seek to replicate and extend findings that AN have a paradoxical response to palatable foods because endogenous DA release in the DC is anxiogenic in AN. This anxiogenic effect is compensated for by restricted eating to avoid anxiety. We will also test the hypothesis that a measure of altered inhibition in AN is associated with DC DA D2 binding.

Behavioral disorders may be caused by complex disturbances in neural pathways. The relationship between altered 5-HT and DA function (and effects on ventral limbic and dorsal cognitive circuits) may modulate pathological feeding behavior in AN and BN. In brief, 5-HT and DA systems are thought to interact (with 5-HT being aversive/inhibitory, and DA modulating reward/motivation) to regulate appetitive processes.[42, 45] Our data[9] showed positive correlations between striatal [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} in REC AN, REC AN-BN, and REC BN. Interactions between [^{11}C]McN5652 BP_{ND} and [^{11}C]raclopride BP_{ND} significantly predicted harm avoidance (HA), a measure of inhibition and anxiety, in REC ED. We hypothesize that binding of these ligands may reflect complex 5-HT – DA neurotransmission interaction processes. In **AIM 3** we seek to replicate these findings and determine whether a similar relationship occurs in CW.

	Limbic DA	**Balance**	**Associative DA**
AN	↓	<	N
AN-BN	↓	Dysregulated	↓
BN	N	>	↓

Table 1 Model of striatal DA functional activity and the balance of limbic versus associative DA striatal circuitry

In **AIM 4** we will explore how [^{11}C]DASB)BP_{ND} and [^{11}C]raclopride BP_{ND} are associated with neural circuits that modulate appetitive behaviors. We will co-register, in the same REC ED and CW, the PET findings in AIMS 1 to 3 with an fMRI study (UCSD IRB project No. 080019; 2 R01 MH0042984-17A1; W. Kaye PI) that examines neural substrates underlying appetitive, as well as reward and cognitive dysregulation. The anterior insula (AI) and striatum integrate the sensory/hedonic aspects of taste, interoceptive awareness, and reward/motivation. Importantly, fMRI studies show that REC AN have diminished[181] and REC BN have exaggerated blood oxygen-level dependent (BOLD) response of this circuitry in response to tastes of sucrose.[134] We hypothesize that restricted eating and weight loss occur in AN because feeding elicits little reward or motivation (due to excessive 5-HT inhibition), whereas the opposite may be true for BN. We predict that REC AN-BN may fall in-between REC AN and REC BN. Our pilot data show that fMRI AI BOLD response to tastes of sucrose was negatively correlated with anterior striatal [^{11}C]raclopride BP_{ND} in REC AN. This supports the speculation that those with the most diminished AI response to sucrose may have the lowest striatal DA concentrations and, thus, reduced appetitive motivation to approach food. In summary, AIM 4 will explore links between striatal [^{11}C]DASB)BP_{ND} and [^{11}C]raclopride BP_{ND}, and neural processes related to appetitive function, reward, inhibition, and self-control. In **AIM 5** (exploratory) we will genotype the 5-HTT, DA D2/D3 receptors, and other relevant genes to explore their relationship with brain imaging.
In summary, this application seeks to understand how [^{11}C]DASB)BP_{ND} and [^{11}C]raclopride BP_{ND} are related to traits that are shared by, or divide EDs into subtypes. Our overarching goal is to characterize neural processes in ED, and their relationship to behavior. Current treatments for AN and BN are of limited efficacy. In **AIM 6** we seek to extend prior animal and patient population work by directly measuring the relationship between spontaneous blink rate and endogenous striatal DA binding. By obtaining average spontaneous blink rates at baseline and during peak AMPH effects, this will be the first direct comparison between experimentally manipulated striatal DA binding and average blink rates in human subjects.

7. BACKGROUND AND SIGNIFICANCE

Background:

AN and BN are disorders of unknown etiology that tend to have their onset during adolescence in women.[1] Although psychosocial factors are hypothesized to cause AN and BN, recent studies show that genetic heritability accounts for approximately 50 to 80% of the risk and creates neurobiological vulnerabilities.[21, 32, 89, 101, 155, 166] It is important to note that considerable evidence has suggested that childhood temperament and personality traits can create a vulnerability for developing AN and BN during adolescence. Recent studies[2, 112, 156] describe negative emotionality, HA, perfectionism, inhibition, drive for thinness, altered interoceptive awareness, and obsessive-compulsive personality traits as childhood risk factors that precede the onset of an ED, persist after recovery, and are elevated in unaffected family members.[33]

Two types of consumatory behavior are seen in AN and BN. Restricting-type anorexics ("AN" in this application) lose weight purely by restricted dieting and have no history of binge eating or purging. Individuals with BN ("BN" in this application) do not become emaciated and are able to maintain an average body weight (ABW) above 85%. BN individuals alternate restricting with episodic binge eating and/or purging. The third category are individuals who have both AN and BN ("AN-BN" in this application). It has been argued that AN and BN share some common risk and liability factors because these disorders are often cross-transmitted in families and share many behavioral traits.[101, 113, 160, 186] Both AN and BN individuals commonly have a seemingly relentless drive to restrain food intake, an extreme fear of weight gain, and a distorted view of their body shape as well as anxiety, obsessionality, and depression. In terms of differences, pure AN tend to be over-controlled, whereas BN and AN-BN tend to have poor impulse control, greater novelty seeking,[112, 159, 179] and high rates of drug and substance abuse.[79]

Neural circuitry of ED: Imaging technology has supported the emerging ability to characterize the neural circuitry of neuropsychiatric disorders.[74] A key element of our research[90] has been to test hypotheses regarding the neural processes that contribute to shared and independent behavioral traits in AN and BN. Work by Phillips et al[137] describes how dysfunction of limbic and cognitive neural networks may occur in a range of

psychiatric disorders. Specifically, a ventral limbic neural circuit, which includes the amygdala, anterior insula (AI), AVS, and ventral regions of the anterior cingulate cortex (ACC) and the prefrontal cortex (PFC), is necessary for identifying rewarding and emotionally significant stimuli and for generating affective responses to these stimuli. A dorsal executive function neural circuit, which includes the hippocampus, dorsal regions of the caudate, dorsolateral PFC (DLPFC), parietal cortex, and other regions, is thought to modulate selective attention, planning, and effortful regulation of affective states. We hypothesize that AN and BN individuals have an imbalance within and/or between ventral limbic and dorsal cognitive circuits. Imaging studies from several groups[169, 180, 182] show that ill and REC AN and BN have altered activity of ventral limbic circuits that play a role in emotionality and reward. Thus, AN and BN may share an inability to precisely identify and/or modulate emotionality and reward in response to salient stimuli. Poor context separation may make them vulnerable to respond inappropriately to the rewarding aspects of appetitive stimuli. In contrast, fMRI studies consistently show that ill and REC AN have increased activity in cognitive neural circuits,[180, 196] whereas ill and REC BN have diminished or impaired activity in these regions;[120, 145, 182] consistent with enhanced higher-order inhibitory function in AN and reduced inhibition in BN. We hypothesize that AN are able to inhibit appetite and have extraordinary self-control because they have exaggerated dorsal cognitive circuit function, whereas BN individuals are vulnerable to overeating when hungry because they have less ability to self-regulate and control their impulses.

Distinguishing Shared and Independent Traits in AN and BN: One goal of this application is to determine how monoamine and neural circuitry contribute to aspects of behavior and may be associated with diagnostic category (e.g., AN vs. BN), perhaps related to impulse control, or aspects that may cut across diagnostic boundaries, such as HA. We will study subjects recruited and assessed by UCSD IRB project No. 080019; 2 R01 MH0042984-17A1. These subjects will have a comprehensive battery of behavioral and cognitive assessments. Still, we want to note that this application will focus on the role of HA, a multifaceted temperament trait[39] that contains elements of anxiety, inhibition, and inflexibility; reflecting the concept of 'behavioral inhibition'. The reason is that 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. HA is positively associated with increased 5-HT_{1A} BP in REC AN,[12] decreased 5-HT_{2A} BP in REC AN-BN,[11], normal 5-HT_{2A} BP in ILL AN/AN-BN[13] and increased DA D2/D3 BP in REC AN/AN-BN.[60] In addition to being a premorbid childhood predisposing factor,[2, 112, 156] elevated HA is common in all ill ED subtypes (for review see Cassin[36]) and elevated HA persists after recovery from AN and BN.[179] This application seeks to understand the 5-HT, DA, and neural circuitry mechanisms contributing to elevated HA in ED subjects. Our data suggest HA is a trait that cuts across diagnostic boundaries (i.e., it is common to AN, AN-BN, and BN). However, it is possible that HA may be associated with extremes of impulse self-control.

Distinguishing State and Trait in AN and BN: When malnourished and emaciated, individuals with AN and BN have alterations of brain and peripheral organ function that are arguably more severe than in any other psychiatric disorder.[50, 87] As a result, neurobiological studies during acute illness are confounded by effects of malnutrition. Determining whether such symptoms are a consequence or a potential cause of pathological feeding behavior or malnutrition is a major methodological problem in the field. Considerable evidence suggests that childhood temperament and personality traits can create a vulnerability for developing AN and BN during adolescence, however, premorbid neurobiological studies in children are not practical. The strategy used in this application is to identify neurobiological vulnerabilities that are independent of the confounding effects of malnutrition by studying women who are REC from AN and BN.[179] Even if persistent physiological disturbances in REC EDs are "scars" they are still likely to help understand the processes contributing to these disorders.

Altered 5-HT function in EDs: It is well known that 5-HT alterations occur in people who are ill with AN and BN and persist after recovery.[30, 88, 185, 192] How 5-HT function contributes to symptoms in AN and BN is not well understood. It is important to note that REC AN and BN have different patterns of 5-HT disturbances (see review by Kaye et al.[88]). For example, different degrees of elevated CSF concentrations of 5-HIAA,[94, 95] reduced 5-HT2A receptor binding,[11, 64, 97] increased 5-HT1A receptor binding[12] and altered behavioral

responses to 5-HT challenges.[63, 99, 149, 188] 5-HT function is known to be inhibitory of appetite and plays a role in anxious and obsessive behaviors, as well as in depression.[23, 40, 78, 110, 117, 119, 151] There is an extensive literature associating 5-HT system activity with fundamental aspects of behavioral inhibition,[71] 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.[52, 147, 190] It is important to note that both AN and BN tend to restrict their eating and lose normal meal patterns.[129] We hypothesize that AN can maintain this inhibition continuously, whereas AN-BN and BN have periodic disinhibition and loss of self-control, resulting in overconsumption of food. Several studies[14, 138] have shown that REC AN and REC BN have differences in 5-HTT function that, in turn, might contribute to extremes of impulse control or inhibition of feeding behaviors. Other evidence of differences in 5-HTT function is that AN have a poor response to SSRIs[6, 34, 53] and other "antidepressant" medication, while these drugs have greater efficacy in BN.

Altered DA function in EDs: Genetic, pharmacologic, and physiological data[20, 66, 88, 96, 109] suggest that ill and REC AN have altered striatal DA function. It remains uncertain whether BN have trait-related DA disturbances because fewer DA studies have been done.[19, 82, 93] In terms of PET studies, our group found that REC AN and AN-BN had increased [^{11}C]raclopride BPND in the AVS.[9, 60] Moreover, [^{11}C]raclopride BPND in the DC was associated with HA in REC AN/AN-BN. DA disturbances in EDs may contribute to an altered modulation of appetitive behaviors, symptoms of anhedonia, dysphoric mood, and increased motor activity.[75, 178] Animal studies indicate that DA in the striatum plays a key role in the optimal response to reward stimuli.[47, 131, 146] To further understand striatal DA pathways and the relationship to reward, our group performed a fMRI study using a monetary choice task known to activate the striatum. Importantly, both REC AN[180] and REC BN[182] failed to have a differential AVS response to positive and negative monetary feedback compared to CW. As noted above, AN and BN may share an inability to precisely identify and/or modulate emotionality and reward in response to salient stimuli.

Exploration of group differences for endogenous DA release in striatal regions of interest (ROIs)

Limbic striatal: Some, but not all, data show that mixed groups of REC AN and AN-BN have elevated baseline AVS [^{11}C]raclopride BP_{ND} compared to CW.[9, 10, 60] While pilot data[10] in a mixed group of REC AN and AN-BN showed a non-significant reduction of endogenous DA release in the AVS, an earlier study from our group showed that only the REC AN (pure restricting-type) had significant reduction of the DA metabolite HVA in CSF compared to CW. In Parkinson's disease there is diminished CSF HVA and extracellular DA.[38, 84] We seek to determine whether pure REC AN (never had BN) have reduced endogenous DA AVS release when compared to CW. Because endogenous DA displaces raclopride at the D2 receptor, elevated DA D2 receptor binding is consistent with diminished DA release. Altered AVS DA function in "pure" restricting-type AN group is likely given that this subtype has substantial anhedonia and reduced consummatory drive and other evidence of dysregulated AVS function.[62, 180, 181]

With regards to predictions of AMPH induced DA release in REC BN and AN-BN, there are limited background data. The pilot data from our group showed a non-significant baseline [^{11}C]raclopride BP_{ND} increase in the AVS in REC BN,[9] but a very large standard deviation (SD). Moreover, REC BN and AN-BN have normal CSF HVA levels[96], and ill BN have normal limbic DA release.[31] Together these data suggest that we will find normal limbic striatal DA release in REC BN. However, most studies to date have investigated mixed groups of AN and AN-BN, or BN and AN-BN. One advantage of the current study is that we are recruiting substantial numbers of "pure" subtypes. For example, the AN have never had BN symptoms. Second, data about DA release are critical for understanding DA's relationship to anxiety and inhibition (AIM 2C) and to building a model (see below) regarding limbic and associative DA balance for the 3 subgroups.

Associative striatal DA predictions: In terms of the DC, the data reviewed above found no difference between REC AN/AN-BN and CW for baseline or Δ BP_{ND} values. We expect to find normal DC baseline [^{11}C]raclopride and Δ BP_{ND} in "pure" REC AN. There are limited data regarding DA function in BN. Broft [31] reported a trend toward decreased mean baseline D2/D3 receptor BP_{ND} in the posterior putamen and caudate in ill BN, and a significantly reduced/blunted DA release after the methylphenidate challenge in the anterior and

posterior putamen. Extrapolating the Broft[31] findings from the ill BN to REC BN may be confounded by state related factors, such as relationship to binge frequency or the effects of malnutrition, as well as mixed diagnostic groups (some of the ILL BN had a history of AN). Still, there are reasons to think (see below) that REC BN will have diminished baseline [^{11}C]raclopride and DA release in the associative and sensorimotor striatum, whether it is a trait or a "scar".[8, 73]

Modeling DA process in AN and BN: AN and BN tend to have opposite extremes of self-control and inhibition. For example, recent meta-analyses[35, 68] show increased rates of drug and alcohol abuse in BN and decreased rates in AN. In a recent review we propose[91] that such inhibitory extremes make BN vulnerable to and AN "protected" from using drugs and alcohol and other consumatory (food) behaviors. There have been relatively consistent findings (reviewed in[176, 177]) of reduced PET [^{11}C]raclopride BP_{ND} in the striatum, including the ventral striatum, in those with substance abuse and alcohol use disorders. Reduced/blunted striatal DA release in individuals who abuse cocaine and alcohol has been exhibited in a smaller number of PET studies in human and primate models.[73, 122, 123, 174] Whether BN and substance abuse share DA D2 receptor related vulnerabilities and whether opposite findings contribute to protective factors in AN remains to be determined.

AIM 2B will contribute to building our model of striatal DA functional activity in AN and BN. Studies of food tastes and negative and positive feedback consistently show altered ventral limbic circuitry in AN and BN (see review by Kaye[91]). In addition, fMRI studies tend to show that AN and BN have altered activity of dorsal executive/cognitive circuitry, which is consistent with the possibility that AN have enhanced higher-order inhibitory function and BN have reduced inhibition. Our overarching goal is to explore the balance between neural processes that modulate reward and inhibition. Do AN have "normal" DA "tone" in cognitive association networks in the dorsal neurocircuit (e.g. DLPFC-to dorsal striatum), which direct motivated actions, but impaired ability of the ventral striatal pathways that direct more 'automatic' or intuitive motivated responses? In BN, is there hypoactivity of dorsal cognitive association pathways that is insufficient in inhibiting "normal" hedonic/incentive motivation impulses in "reward" neural circuits? Alternatively, though not supported by these data, is it possible that AN have adequate ventral limbic-striatal circuitry, but this ventral circuitry is strongly inhibited by "hyperactive" inputs from cognitive domains such as the DLPFC and the parietal cortex? Conversely, BN may have exaggerated hedonic/incentive motivation impulses in "reward" neural circuits that overwhelm normal cognitive inhibition. Predictions about AN-BN are particularly handicapped by lack of data. It may be that they have impairments of both limbic and associated circuits leading to a particularly dysregulated balance between reward and inhibition, or that they have exaggerated defects of associative circuits.

<u>Relationship of endogenous DA release to anxiety and/or inhibition in AN and BN:</u>

Background: Considerable literature (see [36]) shows that HA, a multifaceted temperament trait[39] that contains elements of anxiety, inhibition, and inflexibility, is elevated in individuals who are ill with AN and BN and persists after recovery.[179] As noted above, DC [^{11}C]raclopride BP_{ND} is positively correlated with HA.[9, 60] Importantly, increased DA D2-like receptor functional activity is associated with inhibition in healthy humans.[72] Moreover, rats characterized as risk averse[148] show greater D2 mRNA expression in the DC. In Aim 2C, we seek to explore how DA signaling in associative /executive cortical striatal circuitry may be related to inhibition and/or adverse consequences in AN and BN.

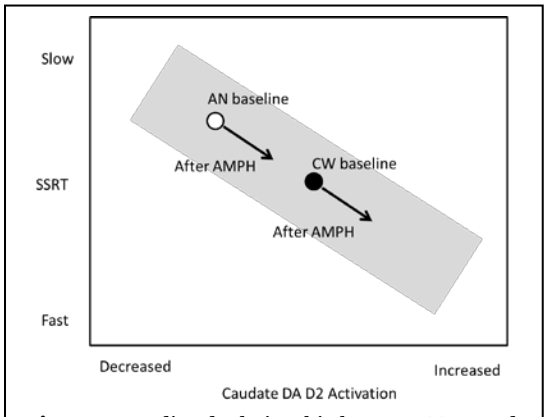

Figure 1: Predicted relationship between SSRT and baseline [^{11}C]racloprideBP_{ND}. Gray shade, range of individual responses; •, mean CW values; O, mean AN values at baseline; →, predicted change when post-AMPH SSRT is compared with baseline [^{11}C]racloprideBP_{ND}.

Anxiety: The major goal in Aim 2c is to replicate and extend our finding that self-reports of HA are associated with baseline D2 binding in the DC in AN and BN, and self-reports of anxiety are

associated with endogenous DA release in the DC in AN. Our pilot data in REC AN[10] show AMPH stimulated anxiety and this was positively associated with the magnitude of DA release in the DC. This is different from studies in controls, who tend to become euphoric after AMPH in association with AVS stimulant-induced striatal DA release.[49, 107, 121, 175] Most people find eating to be a pleasant and rewarding experience. But for AN, eating stimulates dysphoric mood, while there is an anxiety-reducing character to dietary restraint and reduced caloric intake.[61, 86, 153, 157, 173] Ingestion of palatable food is associated with striatal endogenous DA release.[8, 17] We hypothesize that food-induced release of DA activates DC D2 receptors resulting in anxiety in AN (and not AVS related reward and motivation as in healthy controls). As a result, AN individuals pursue starvation because food refusal may be a way of diminishing DA release and concomitant anxiety. In fact, AN individuals have decreased CSF HVA, the major metabolite of DA,[92, 96] supporting this hypothesis. Predicting response in REC BN and AN-BN is more speculative. We may find a heterogenous response to AMPH in REC BN and AN-BN. In those who have high HA traits, we expect to find that AMPH administration makes them more anxious, and that anxiety will be associated with increased endogenous DA in associative and sensorimotor striatal regions. There may also be a group that becomes euphoric on AMPH. Broft[31] reported evidence of an association between striatal DA response and ratings of "energetic" in ill BN but these were said to be driven by a small number of data points. This group, which may be at higher risk for substance abuse, may have diminished endogenous DA in associative regions, or enhanced AVS DA release associated with pleasure.

Inhibition: Clinically, it is well known that AN have exaggerated self-control and are anhedonic so they are able to sustain self-denial of food as well as most comforts and pleasures in life.[60] For example, AN have an enhanced ability to delay reward (i.e., show less reduction in the value of a monetary reward over time) compared to healthy volunteers.[154] This enhanced cognitive control and ability to delay reward may help to maintain persistent food restriction. We will build on a recent study[72] in healthy controls that showed that striatal (particularly in dorsal regions) DA D2/D3 receptor availability was negatively correlated with speed of response inhibition [stop signal reaction time (SSRT)] and positively correlated with inhibition-related fMRI activation in frontostriatal neural circuitry. Thus people with faster stopping speed had more D2 receptor binding in the caudate and putamen. The stop signal task (SST) requires people to stop an already initiated response[5] and calculates the speed of stopping (SSRT). There are relatively little response inhibition data (with SST or any other task) in AN, but a recent study in AN found that they had longer SSRT.[69] Our data[9, 60] show that AN have normal DC D2 binding. However, as noted above, there is evidence that ill[92] and recovered AN[96] have reduced endogenous DA. Because AN may have less DA activating the DA D2 receptors, this may explain why their average inhibition ability is impaired. We will administer the SST after subjects complete the baseline [^{11}C]raclopride PET study. We predict (**Figure 1**) that both AN and CW will show a negative relationship between SSRT and DC D2 binding, but that such a relationship in AN will be shifted to the left due to reduced DA activation of the D2 receptor. While slower inhibition in AN may seem counter-intuitive, this finding may be consistent with studies that show they have impaired abilities to change mental set.[144]

We will repeat the SST after administration of AMPH. Studies tend to show[46, 111, 142] that AMPH improves inhibition in healthy people without changing "going" speed. That is consistent with the idea that AMPH induced release of endogenous DA increases activation of D2 receptors and thus underlies inhibition-related caudate activity.[72] We expect to find that AMPH shifts healthy controls to the right (**Figure 1**). An abnormal shift to the right in AN would suggest aberrant function of DA/D2-related processes. It is possible that higher anxiety in AN[179] contributes to the slower stopping speed. While low threat puts people in a state of arousal where they can stop things more quickly, high threat association appears to make them slower.[136] We will stratify our AN population into high and low state anxiety groups to better understand the relationship between anxiety and response inhibition ability in this group, and how that relates to the AMPH challenge and a putative marker of endogenous DA. Overall, too few data exist to be certain that the SST is an adequate reflection of inhibitory control ability in AN (especially in a way that relates to self-control). Individuals with BN and AN-BN have a puzzling group of symptoms in that they alternate between being overly inhibited and overly disinhibited: eating behaviors tend to alternate between restriction and overeating with a loss of normal meal patterns.[189] This study will provide the opportunity to better understand relationships between [^{11}C]raclopride

BP_{ND} or DA release and measures of inhibition and anxiety in BN and AN-BN.

Significance: The understanding of the pathophysiology of EDs has tended to lag behind other major psychiatric disorders. Improvement in the understanding and treatment of EDs are of immense clinical and public health importance[133] as these are often chronic, relapsing illnesses[77, 100, 102] with substantial and costly medical morbidity,[124] and a high mortality.[161] AN and BN have puzzling symptoms that are unique to the disorders: extremes of eating (restricting, bingeing/purging), relentless drive to lose weight, body image distortions, denial of illness, etc. We have little understanding of how such symptoms are encoded in the brain. These symptoms appear to be unique to humans. Since animal models have not been developed displaying these puzzling symptoms, studies in humans are essential. The overarching goal of this, and related applications, is to use imaging to understand the neural circuitry of AN and BN and how symptoms are encoded in these circuits.

Importantly, for AN, there is no proven treatment that reverses symptoms[133] or FDA approved medication.[7, 34, 83] While those with BN may respond better to treatment, many continue to be symptomatic or relapse.[184] Despite the evidence of 5-HT and DA disturbances in ED, there is little evidence that selective 5-HT reuptake inhibitors (SSRIs)[6, 34, 53] or the neuroleptics pimozide and sulpiride[170, 171] stimulate weight gain or reduce core symptoms in ill AN. Moreover, it remains controversial as to whether SSRIs reduce relapse after weight gain.[98, 158, 183] Still, preliminary data suggest that atypicals, such as olanzapine,[15, 22, 24, 26, 28, 48, 51, 76, 81, 105, 118, 127, 130, 140, 187] quetiapine,[27, 126, 139] risperidone,[58, 132] and aripiprazole[168] may be useful in reducing anxiety and promoting weight gain in ill AN. Many of the psychoactive medications that are approved for use in humans act on 5-HT and DA pathways. Thus, a better understanding of how 5-HT and DA contribute to symptoms in ED may help us advance beyond the trial and error system and develop new methods for identifying effective medications and psychological treatments.

Relationship between spontaneous blink rate and striatal dopamine

The hypothesized relationship between spontaneous eye blink rate and striatal DA has been around for the last several decades. Experimental evidence supporting this hypothesis exists almost exlusively in animal research[85, 165] and in patient populations[85] despite being used as an established measure during tasks[25]. Using pharmacological[85, 103] and neurotoxic[165] manipulations of the DA system in monkeys, a strong case has been made for the role of striatal DA in moderating spontaneous blink rates with striatal DA levels being positively correlated with spontaneous blink rates. This link has been made using direct, and indirect DA agonists in Cynomolgus monkeys[103], and was further refined by isolating the caudate as a potential key source of this link using the dopaminergic neurotoxin MPTP in monkeys (cercopithecus aethiops sabaeus). By using the well established dopaminergic abnormalities in schizophrenia and Parkinson's Disease[85], research in these patient populations have indicated that these DA abnormalities seem to be inducing the expected changes based on each disease's dopamine profile consistent with the animal literature. This work seeks to extend past patient and animal research by being the first to directly compare and experimentally manipulate the relationship between striatal dopamine binding and spontaneous blink rate.

8. PROGRESS REPORT

To date a total of 47 participants have been enrolled into the study. We have successfully completed a total of 46 [^{11}C]DASB (in 18 CW, 11 REC AN, 10 REC AN-BN, 7 REC BN) and a total 47 [11C]raclopride scans (in 19 CW, 11 REC AN, 10 REC AN-BN, 7 REC BN).

9. RESEARCH DESIGN AND METHODS

Screening and Assessments Prior to Imaging Studies:

Once the written consent has been obtained, subjects will complete screening interviews (for Axis I and II diagnoses, including ED subtype diagnosis), a physical examination (15-20 min), and laboratory tests, e.g., EKG,

VS, hemoglobin, hematocrit, total leukocyte with differential, plasma electrolytes, liver and thyroid function (T3, T4 and TSH levels), urinanalysis, toxicology and confirmation of toxicology results (if required), cortisol levels, estradiol levels and pregnancy test prior to entering the study as previously described.[12, 179] The measures used in this study include the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) to assess the lifetime prevalence of Axis I psychiatric disorders, a modified version of Module H of SCID-I to assess lifetime diagnoses of EDs and subtypes and the Yale-Brown Obsessive Compulsive Scale for the assessment of OCD symptoms. Subjects will self-report ED symptoms, depression, anxiety, impulsivity, perfectionism, and temperament.[16, 18, 41, 67, 70, 152, 162] To reduce confounding influences, we will exclude ED subjects who, in the past 3 months, have been on psychoactive medication or met criteria for substance abuse/dependence, major depression, or an incapacitating anxiety disorder.

Subjects will also complete behavior self-ratings including assessments of personality, mood, depression, anxiety, impulsivity and other behaviors relevant to eating disorders (See Table 2 for complete listing). These assessments will be sent to their home prior to scheduling them for an interview. The dominant hand will be assessed by the Edinburgh Inventory for analysis of handedness.

Table 2: Interviews and self-assessments

Name	Abbreviation	Area assessed	Minutes
Interviews:			
Mini-International Neuropsychiatric Interview	M.I.N.I. Plus	Axis I diagnoses	60
Module H of SCID I, Modified	Module H	ED diagnoses	30
Yale-Brown Obsessive Compulsive Scale	Y-BOCS	OCD	30
Yale-Brown-Cornell Eating Disorder Scale	YBC	ED symptoms	15
Set-shifting	SS	Inflexibility	5
Self-Assessments:			
Beck Depression Inventory	BDI	Depression	10
Spielberger State-Trait Anxiety Scale-Version Y	STAI-Y	State and trait anxiety	10
Eating Disorders Inventory -2	EDI-2	Core ED symptoms	15
Multi-dimensional Perfectionism Scale	MPS	Perfectionism	10
Barrett Impulsivity Scale	BIS	Impulse control	10
Temperament and Character Inventory	TCI	Personality and temperament	30
Sensation Seeking Scale	SSS	Sensation Seeking	15

Study-Day Session Procedures: Each study will be done during the early follicular phase (days 1 to 10) of the subject's menstrual cycle. Subjects will arrive the day before the study and stay overnight at the Clinical and Translational Research Institute (CTRI) at UCSD. On the day of the study, they will be awakened at 6:00 A.M. by the research staff. After a light breakfast, they will be transported by taxi to the Keck Center for Functional Magnetic Resonance Imaging on the UCSD campus at 7:00 A.M. After the acquisition of the structural MRI, which will be completed in about 30 minutes, the subjects will be transported again by taxi to the UCSD NeuroPET Center. The subjects will be informed of all of the procedures that will be performed and the information that will be obtained. The subject confirmation will be made via the signed copy of the written informed consent and then an intravenous catheter will be placed in either the left or right antecubital vein. After insertion of an intravenous catheter in a forearm vein, and a 20 minute transmission scan, a PET scan will be acquired following a slow bolus administration (over 20 sec) of 15 mCi of high-specific activity [^{11}C]DASB. Emission data will be collected in 3D mode for 100 minutes using a standard PET protocol as previously

described.[65] At the completion of the scan, the IV lines will be removed. An EKG, vital signs, mental status and physical exam will be performed and evaluated by the study physician prior to the subject's discharge from the PET suite.

On the following day, the second day of the study, they will be awakened at 7:00 A.M. by the research staff. After a light breakfast, they will be transported again by taxi to the UCSD NeuroPET Center. After insertion of another intravenous catheter and following another transmission scan, the first PET scan will be acquired following a slow IV bolus administration (over 20 sec) of 10 mCi of high-specific activity [^{11}C]raclopride. Emission data will be collected in 3D mode for 60 minutes using a standard PET protocol as previously described.[60] The participant will then be able to rest outside the scanner and will receive a physical exam, mental status exam, EKG, and vital signs measurement. The IV will stay in place. Thirty minutes following the first scan with [^{11}C]raclopride, the participant will receive 0.5 mg x kg-1 of oral AMPH. Blood pressure and heart rate will be recorded every two minutes for the first 15 minutes, every five minutes for the next 30 minutes, and then every 15 minutes (up to at least 180 min). The post-AMPH [^{11}C]raclopride scan will be performed 3 hours after the administration of AMPH. Assessment of behavioral responses will be conducted before and after PET scans. The participants will complete a symptom-rating visual analog scale (VAS) pre-baseline PET, pre-AMPH, and then at 30 minute intervals a questionnaire assessing several states: "happy," "anxious," "energetic," "restless."[163] To assess study participants' mood states and fluctuations, we will utilize, at the same intervals, the short version of Profile of Mood States (POMS-B).[125] The POMS is a useful measure of mood states and their fluctuations and is appropriate for both research and therapy. It is a factor-analytically derived self-report inventory that measures six identifiable mood or affective states: tension-anxiety; depression-dejection; anger-hostility; vigor-activity; fatigue-inertia; and confusion-bewilderment. For more than 30 years, the POMS assessment has provided a valid and reliable measure of affective mood states. The short version, POMS Brief, developed in 1989, has a total of 30-items with five on each of the six POMS factors. Like the longer version, the POMS-B has excellent internal consistency. Items are rated on a 5-point scale (not at all, a little, moderately, quite a bit, extremely) and they only take five minutes to compete. The items are easy for patients to understand, allowing them to quickly and accurately complete the assessment. The basic stop signal task[115] will be done after the 1st [^{11}C]raclopride (at baseline) and then shortly after the 2nd [^{11}C]raclopride (post-AMPH) scan. The study will be completed by approximately late afternoon. At the completion of the second scan, the IV lines will be removed. An EKG, vital signs, mental status and physical exam will be performed and evaluated by the study physician prior to the subject's discharge from the PET suite. The subject will be served a cafeteria-style assortment of lunch foods following the [^{11}C]DASB scan on Day 1 and the post-AMPH [^{11}C]raclopride scan on Day 2. The uneaten food will be weighed after completion of the meal and the total calories consumed will be recorded.

MRI Protocol: All participants will undergo an MRI procedure for anatomical localization of ROIs. Only one MRI will be done per participant. A single MRI study will be performed at the UCSD Keck Center for Functional MRI using a GE Excite HDx 3.0 Tesla scanner as described in our parent grant. The registered MRI will be used as an individualized anatomic map for the selection of ROIs used in the analysis of the PET data (see section Neuroimage Analysis).

Radiochemistry of [^{11}C]raclopride and [^{11}C]DASB:

[^{11}C]raclopride will be prepared at the UCSD Center For Molecular Imaging, PET Radiochemistry Laboratory. Radioactive [^{11}C]CO2 will be produced by the 14N(p, α)^{11}C nuclear reaction at the CTI RDS 111 cyclotron operated by PETNET. The [^{11}C]CO2 will be converted into [^{11}C]CH3I in GE Tracerlab FX MeI box sequentially swept into a silver triflate oven to produce [^{11}C]methyl triflate. O-desmethylraclopride tetrabutylammonium precursor that is reacted using the HPLC loop injection port procedure. Then, the contents of the loop will be quantitatively injected into a semi preparative liquid chromatography column. The [^{11}C]raclopride fraction will be isolated, passed through a 0.22 μm sterile Millex-GV membrane filter, and collected in a 30 mL sterile vial already containing 15 mL of 0.9% sterile saline. Quality Assurance will be

performed as recommended by the FDA. For [^{11}C]raclopride the radiochemical yield is 30-40% at the end of synthesis. The total synthesis time will be 40± 5 min. The specific activity at the end of the analysis is 1500 Ci/mmol to 4000 Ci/mmol. The studies are carried out under IND 74,250.

[^{11}C]DASB will be prepared at the UCSD Center For Molecular Imaging, PET Radiochemistry Laboratory. Radioactive [^{11}C]CO2 will be produced by the 14N(p, α)^{11}C nuclear reaction at the CTI RDS 111 cyclotron operated by PETNET. The [^{11}C]CO2 will be converted into [^{11}C]CH3I desmethyl-DASB precursor that is reacted using the loop injection port procedure. Then, the contents of the HPLC loop will be quantitatively injected into a semi preparative liquid chromatography column. The product will be transferred into a flask containing a multiple volume (10x with respect to the product peak volume) of HPLC water. After that the aqueous solutionis pushed through a solid phase extraction cartridge containing C-18 (usually WATERS SepPak light C-18 cartridge). The product is retarded on the C-18 cartridge. The cartridge is washed with 10 ml sterile water followed by product extraction with 1 ml USP EtOH transferred into a sterile product vial already containing 19 ml isotonic saline using a 22 μm sterile Millex-FG membrane filter. Quality Assurance will be performed as recommended by the FDA. The total synthesis time will be 50± 5 min. The average specific activity at the end of the analysis has averaged 1000 to 5000 Ci/mmol. The studies are carried out under IND 74, 254 for [^{11}C]DASB.

Dextroamphetamine sulfate (oral) is the dextro isomer of the compound d,l-AMPH sulphate. It is an FDA approved drug available for the treatment of narcolepsy and attention deficit hyperactivity disorder (maximum approved total daily dose of 5-60 mg). It is manufactured and marketed under the commercial name Dexedrine by GlaxoSmithKline pharmaceuticals. As dexedrine is available as an orange, triangular, scored tablet in 5 mg, 10 mg and 15 mg strengths, it will be dispensed by the pharmacy as nearly as it can be approximated to 0.5 mg/kg (in 2.5 mg increments). For example a subject who weighs 50 kg or 54 kg will receive 25 mg of AMPH, while a subject who weighs 55 kg will receive 27.5 mg of AMPH. The use of AMPH in this study will be in an off-label manner. The pharmacokinetics and behavioral effects of oral dextroamphetamine peaks at 3h.[104] Several other groups have successfully used oral AMPH in the dose range of 0.43-0.5 mg/kg in PET imaging studies.[10, 143, 191, 193, 194]

PET scan protocol: The PET imaging will be done at the UCSD NeuroPET Center using a HR+ PET scanner under the supervision of Drs. Hoh and Buchsbaum. An intravenous catheter will be placed in a forearm vein for radiopharmaceutical infusion (this protocol will not involve arterial catheterization) and collection of blood samples. The subject's head will be positioned and immobilized using a vacuum bead padding to decrease head movement during the scan. A 20 minute transmission scan, using rotating rods of Ge-68/Ga-68, will be obtained for attenuation correction of the emission scan(s). On the first day of the study, after the transmission scan, a PET scan will be acquired following a slow bolus administration (over 20 sec) of 15 mCi of high-specific activity [^{11}C]DASB. Emission data will be collected in 3D mode for 100 minutes using a standard PET protocol as previously described.[65] At the completion of the scan, the IV line will be removed. On the next day, following another transmission scan, the first PET scan will be acquired following a slow IV bolus administration (over 20 sec) of 10 mCi of high-specific activity [^{11}C]raclopride. Emission data will be collected in 3D mode for 60 minutes using a standard PET protocol as previously described.[60] Blood pressure and heart rate will be recorded every two minutes for the first 15 minutes, every five minutes for the next 30 minutes, and then every 15 minutes until the subject is placed in the PET scanner (up to at least 180 min). The second [^{11}C]raclopride scan will be done 3 hours post AMPH and will require a second PET transmission scan. During the waiting period (post AMPH), the participant will be asked to perform a few structured tasks and rating scales such as the Visual Analog Scale (VAS) and Profile of Mood Scale (POMS)[125] to contrast with baseline. Approximately 5 mL of venous blood will be drawn at 0 and 30 min relative to the second [^{11}C]raclopride scan to measure plasma AMPH levels. Following each PET scan, an EKG, vital signs, mental status and physical exam will be performed and evaluated by the study physician prior to the participant's discharge from the UCSD PET center and the IV line will be removed.

Stop Signal Task: The basic stop signal task[115] will be done after the 1st [^{11}C]raclopride (at baseline) and then shortly after the 2nd [^{11}C]raclopride (post-AMPH) scan. The test-retest reliability of the task is high.[150] The task has been implemented in Matlab and Psychtoolbox 3,[4] and can be run in 15 minutes, including practice. SSRT, the main dependent measure, will be computed using an approach that is optimal for treating between-group differences.[172]

Neuroimage Analysis

MRI-Based Region of Interest (ROI) Definition for analysis of PET Data**.** PET and MRI image sets are aligned using PET/MRI fusion software (IDL, ITT Visual Information Solutions). ROIs are defined on MRI images across all MRI slices passing through those structures using an ROI drawing tool (imagetool) by trained tracers (blind to the diagnosis) with established reliability. For the [^{11}C]raclopride and [^{11}C]DASB studies ROIs will include the three functional sub-divisions (limbic, associative and motor) of the striatum as outlined by Martinez *et al.*[121]. The limbic striatum comprises the AVS; the associative striatum comprises the pre-commisural DC, precommisural dorsal putamen and postcommisural DC; the motor striatum comprises the post commisural dorsal putamen. For the [^{11}C]DASB studies, as outlined by Frankle *et al.,*[65] The subcortical regions include thalamus, insula, midbrain, medial temporal lobe (a spatially weighted average of 5 limbic structures, the uncus, amygdala, entorhinal cortex, parahippocampal gyrus, and hippocampus). The neocortical ROIs are as follows: DLPFC, medial PFC, OFC, ACC, temporal and occipital cortex. The cerebellum will be used as reference region for both studies. A different set of ROIs are routinely utilized for fMRI studies. As a result, we will define a second ROI set that will be applied to the PET BP_{ND} maps and enable us to perform the integrative analyses to relate the PET and fMRI data in exploratory AIM 4.

Anatomically constrained functional regions of interest for fMRI analyses. One of our co-investigators, Dr. Anders Dale, in collaboration with his colleagues, has developed methods for reconstructing the cortical sheet based upon routine 3D T1-weighted MRI volumes.[43, 44, 54, 55] FreeSurfer not only allows for the accurate measurement of cortical thickness, it also provides a method for parcellation of the cortical surface, where each location on the surface is assigned a neuroanatomical label based upon probabilistic information estimated from a manually labeled training set.[56] The ROIs that will also be used for fMRI analysis include: bilateral caudate, ventral striatum, medial PFC, ACC, DLPFC, medial OFC, inferior frontal gyrus, inferior parietal cortex, and AI.

Analysis of PET Data: The "PET" ROIs will be applied to the dynamic [^{11}C]raclopride and [^{11}C]DASB images to obtain decay-corrected, time-radioactivity curves using a calibrated phantom standard to convert tomographic counts to μCi/ml for each time point. Analysis of the PET data for both [^{11}C]raclopride and [^{11}C]DASB will be performed using both the simplified reference tissue[106] and graphical reference tissue[116] methods with the cerebellum as reference region for non-displaceable uptake. Both of these reference tissue methods have been shown to be appropriate models for quantifying [^{11}C]raclopride[49, 60] and [^{11}C]DASB binding in humans[65, 164] without an arterial input function. The outcome measure derived from these analyses is BP_{ND}. BP_{ND} (unitless) is equal of $f_{ND}B_{avail}/K_D$ where B_{avail} is the concentration of receptors available for radioligand binding *in vivo* (not bound to endogenous ligand), K_D is inversely related to the *in vivo* affinity of radioligand for receptor binding and f_{ND} is the fraction of radiotracer freely dissolved in tissue water (i.e. free fraction) in the non-displaceable compartment.[80] We will also generate parametric BP_{ND} maps that will be spatially normalized to the MR data set and the "fMRI" ROIs will then be applied to sample the normalized PET BP_{ND} maps.

FC analysis across regions. We will explore functional inter-relationships in AI, OFC, striatum, and ventral and dorsal putamen by combining information from both fMRI and PET data. Initial analyses will assess for outliers and distributions of fMRI and PET data times series to ensure modeling assumptions are met. Outliers will be Winsorized; non-normality will be handled via appropriate transformations. Missing data will be multiply imputed.[114] FC of activation in these regions in response to sucrose stimulus will be assessed on an individual subject-level basis via partial cross-spectrum of BOLD activation time series.[197] The partial cross

spectrum will be used to estimate the normalized partial mutual information, an overall measure of the linear and nonlinear FC between pairs of regions controlling for activations in the remaining regions. The stimulus waveform will also be entered as a separate time series in this model to control for non-stationarity in activation of regional BOLD time series due to stimulus. The resulting FC estimates will then be entered into a random effects model with clustering of responses by subject; subject-level PET 5-HT/DA function in these same regions will be entered along with group membership (REC AN, REC AN-BN, REC BN, and CW). This random effects model will be used to test for whether any group differences in FC in this network are (partially) explained by subject differences in 5-HT and/or DA function. The methods implemented in the FC toolbox in MATLAB (Version 7.6)[197] will be adapted to perform these analyses.

Exploratory analyses of PET and fMRI data relationships. Relationships between the PET BP_{ND} and Δ in BP_{ND} values and the fMRI results will be explored with supplemental statistical analyses, performed across subjects. First, task-specific BOLD fMRI activation will be related to a specific PET BP_{ND} value following the methods of Fisher et al. (2009).[57] A partial least squares (PLS) multivariate analysis will be applied to relate regional results and/or voxel level contrast maps.[37, 141, 195] PLS is a non-parametric dimension reduction method that will utilize 2 blocks of manifest variables (X and Y) that can be statistical contrasts, covariates or neuroimaging data. The inter-block covariance matrix R_{YX} between X and Y is determined and primary outcomes are scores that represent the elements of block-X and block-Y that are most correlated. PLS can be applied to relate fMRI single-subject ROI results to PET BP_{ND} maps or relate a difference map for a fMRI BOLD contrast to a difference map for a PET BP_{ND} contrast between groups.

Genetic Analyses:

A one-time 20cc sample will be collected on the first scanning day only from consenting participants for the purpose of extracting DNA to determine whether polymorphisms of physiological systems that are known to influence 5-HT and DA function contribute to binding on the PET/radioligand studies with women who have an eating disorder history. All samples collected will be identified without personal identifiers, but coded, and known only by Dr. Kaye's research team. Genetic samples will be used for eating disorder research, obesity or related disorders. Should a participant withdraw her consent from this genetic testing, the samples will be immediately destroyed. Otherwise, frozen samples will be kept under locked conditions at UCSD in the Eating Disorders Center for Treatment and Research for an indefinite time where they will be submitted for analysis with DNA and lymphocyte extraction. A specific consent for this blood draw will be signed at the time of study consenting if the participant is interested in participating. Blood samples for genetic analysis will be stored in freezers at the Eating Disorders Center for Treatment and Research under supervision of Dr. Kaye. Dr. Kaye will be responsible for determining how DNA will be used. The specimens collected and the DNA that they contain may also be used in additional research to be conducted by the University of California personnel collaborating in this research. In addition to Dr. Kaye, subjects DNA may also be studied by other future collaborators that are unknown at this time to learn more about eating disorders, obesity and other related disorders. No more than a total of 105 cc of blood will be drawn for both studies together; this amount is approximately 20-25% of a regular blood donation.

Power and Statistical Analysis Plan:

Aim 1: In AIM 1 we will use PET and [^{11}C]DASB BPND to determine whether there is a continuum of 5-HTT binding associated with diagnosis. We hypothesize that REC AN will show increased and REC BN decreased [^{11}C]DASB BPND in midbrain and striatal regions. REC AN-BN will have [^{11}C]DASB BPNDs between those of REC AN and REC BN and will probably not differ from CW. These hypotheses will be tested by an unpaired t-test with diagnosis as factor and [^{11}C]DASB BPND as dependent variable. We will use Troendle's (1995) method to adjust for multiple comparisons.[167] Our preliminary study[14] found [^{11}C]McN5652 BPNDs of 1.169 (CW), 1.316 (AN), 1.027 (BN), and 1.168 (AN-BN) in the dorsal raphe, with a SD of 0.348 or less in each group. [^{11}C]McN5652 is an older radioligand and, based on additional pilot data, we expect to see [^{11}C]DASB BPNDs that are roughly 2.17-times higher than [^{11}C]McN5652 BPNDs in the midbrain, with only a

slight increase in within-group SDs (less than 0.382 for [^{11}C]DASB BPND). With 25 subjects per group, a (Welch-Satterthwaite) t-test will have power in excess of 80% to see a difference between the REC AN and CW (based on a mean difference of 0.319 and a within-group SD of 0.382) and REC BN and CW (based on a mean difference of 0.308 and a within-group SD of 0.382) at the 0.05 level (two-sided). We expect the [^{11}C]DASB BPND for REC AN-BN to be between that of REC AN and REC BN and (essentially) the same as CW.

Aim 2A will compare REC AN, REC AN-BN and REC BN to CW to test the hypothesis that REC AN and REC BN have increased AVS [^{11}C]raclopride BPND compared to CW, and to further explore [^{11}C]raclopride BPND in REC AN-BN. We will assess [^{11}C]raclopride BPND in ventral and dorsal putamen, ventral and DC and consider the influences of age, weight, and other relevant variables. These hypotheses will be tested by an unpaired t-test, with diagnosis as factor and [^{11}C]raclopride BPND as dependent variable. We will use Troendle's (1995) method to adjust for multiple comparisons.[167] Based on our pilot data we expect to see [^{11}C]raclopride BPND of roughly 2.120 (CW), 2.404 (REC AN), 2.299 (REC BN), and 2.147 (REC AN-BN) in the AVS, with SDs of roughly 0.359 in each group. With 25 subjects per group, a (Welch-Satterthwaite) t-test will have power in excess of 90% to detect differences in [^{11}C]raclopride BPND between each of the ED groups and CW at the 0.05 level (two-sided). We will also regress log [^{11}C]raclopride BPND on group (REC AN, REC AN-BN, and REC BN, with CW as the reference group), controlling for age, height, and weight. To the extent that these additional covariates are associated with log [^{11}C]raclopride BPND, this will reduce the standard error associated with each of the group effects leading to improved power.

In **Aim 2B** ee hypothesize DA release (as reflected by AVS Δ BP_{ND}) will be diminished in REC AN patients when compared to CW. This hypothesis will be tested using linear regression, with diagnostic group as factor and log AVS BP_{ND} as dependent variable, controlling for age, height, and weight. For Aim 2B, we propose a regression model of the form: [log(post-AMPH BP_{ND}) = a + b * log(baseline BP_{ND}) + c * group + error and hypothesize that the group differences (c) will be statistically significantly different from 0. Indeed, based on our preliminary data, we expect a pre-post difference in log-binding-potential [log(BP_{ND})] in the AVS of 0.132 in the REC AN group and 0.101 in the CW with SDs of 0.048, corresponding to a group difference of c = 0.031, with a pre-post correlation of 0.90. Assuming this, with 20 subjects per group, we will have 51% power at the 0.05 level (two-sided). This is improved by controlling for age, height, and weight. Indeed, controlling for these produces a smaller error SD of 0.034, with the same coefficient estimates as above. In this case, we will have power in excess of 0.80 for group differences at the 0.05 level (two-sided). Preliminary data from Broft et al.[31] suggest the differences between ILL BN and CW in Δlog(BP_{ND}) are even larger post-pre methylphenidate administration. Indeed, they saw changes on the order of 2.69 on the log scale for ILL BN and 3.17 for CW in the putamen with SD below 2.53. This corresponds to a group difference of 0.471 and, assuming similar pre-post correlations and effects of age, height, and weight, we should have power greater than 0.80 to see group differences at the 0.05 level (two-sided) in this case as well.

Aim 2C Relationships between [^{11}C]raclopride Δ BP_{ND} and behavioral characteristics (from assessments and VAS scales) and plasma AMPH levels of subjects will be analyzed with Pearson Product-Moment correlation coefficient. For example, we will compare the Fisher's z-transformed correlations between behavioral measures of euphoria and anxiety with [^{11}C]raclopride Δ BP_{ND} in REC AN, REC AN-BN and REC BN vs. CW using a t-test. Based on our pilot data in REC AN, we expect correlations between euphoria and [^{11}C]raclopride Δ BP_{ND} in the AVS to be larger (in magnitude) than -0.69 in CW and 0.21 in REC AN subjects. Assuming a difference in Fisher's z-transformed correlations of 1.06 (or larger), we will have power 0.91 to see a group difference at the 0.05 level (two-sided), with 20 subjects per group. (Note that the z-transformed value of 0.69 is -0.85, and the z-transformed value of 0.21 is 0.21, giving a difference of 0.21 - (-0.85) = 1.06). Similarly, we will correlate DA release with self-ratings of anxiety, euphoria, self-control, and labile mood in striatal regions in order to assess behavioral response, as well as SSRT and post-study food consumption. We will use continuous

measures of impulse control and inhibition including the SSS,[198] BIS-11,[16] EDI-2-subscale impulse regulation,[70]novelty seeking and HA from the TCI[41] and the basic stop signal task.[115] It is not certain what to expect from the REC BN and REC AN-BN subjects, but we will look for differences in correlations in the same way. Assuming these differences are larger than 0.909, we will have power in excess of 0.80 to see group differences at the 0.05 level (two-sided). We will explore the effects of age, height, and weight on DA release using partial correlations, after controlling for these variables.

Spontaneous Eye Blink Rate: Collecting of eye blink rate data will be done two times during the study, shortly before the 1st [^{11}C]raclopride scan (baseline), and shortly before the 2nd [^{11}C]raclopride (post-AMPH) scan. Spontaneous eye blink rate will be measured by having participants sit in front of a computer screen that is equipped with a video recording device. Participants will be told to look at the fixation cross in the center of the screen for a duration of five minutes with the explanation that we are interested in recording aspects of facial movements. Participants will not be told that blink rates are the object of the fixation to ensure that blink rates truly are spontaneous and are not being intentionally manipulated by the participant. Data will subsequently be scored by averaging across each five minute span to obtain a blink per minute rate, similar to prior work using this methodology.[85]

10. HUMAN SUBJECTS

Subject Population: We seek to recruit 30 REC AN, 30 REC AN-BN, 30 REC BN, and 30 CW, between ages 18 to 45 for the PET imaging studies. It is our experience from previous studies that it will be necessary to enroll an additional 20% of subjects due to drug failure, non-completion of PET studies, etc. Thus, we expect to complete PET studies in a total of 100 subjects (25 REC AN, 25 REC AN-BN, 25 REC BN, and 25 CW).

Only right-handed subjects will be included as screened by the Edinburgh Handedness Inventory.[135] The rationale for including only female subjects is that AN, AN-BN and BN among males is relatively rare (less than 10% in our experience). Furthermore, because some males with AN, AN-BN or BN have many atypical features, they should be subjects of a different study. All subjects will be matched by age, IQ, income, race/ethnicity, and will be non-smokers. REC AN, REC AN-BN, REC BN, and CW will be matched by BMI. Subjects will be recruited locally and nationally to reflect the racial/ethnic composition of the United States, which encompasses a wide range of ethnically diverse individuals (i.e., 65.6% White persons, not Hispanic, 15.4% Hispanic or Latino origin, 0.2% Native Hawaiian and Other Pacific Islander, 4.5% Asian persons, 1.0% American Indian and Alaska Native persons, 12.8% Black persons, 79.8% White persons (http://quickfacts.census.gov/qfd/states/00000.html)**.** While every effort will be made to recruit participants in respective proportion to these demographics, it has been our experience that patients with AN, AN-BN and BN tend to be predominantly white. Subjects fulfilling all the inclusion, but none of the exclusion criteria, will be admitted to the study. No exclusion criteria shall be based on race or ethnicity.

Inclusion Criteria for REC AN, REC AN-BN and REC BN Women**:** REC AN, AN-BN and BN subjects must have, at some point in their life, met a DSM-IV diagnosis of AN and/or BN. The onset of their illness must have been at least 4 years prior to participating in this study. Due to the high rate of conversion from AN to BN within 3 years of onset, this time requirement will ensure that they did not convert from one diagnostic subtype to another. Thus, diagnostically "pure" groups may be obtained. REC AN women must have met DSM–IV criteria for AN, restricting type, with an ABW below 85% for height.[128] They must never have engaged in binge eating (as defined in DSM–IV) or purging behaviors (e.g., vomiting, laxative or diuretic abuse, enemas, etc.). REC AN-BN subjects must, at some point in their life, have been below 85% ABW and have had binging/purging behaviors during a period of low weight. REC BN must have met a lifetime DSM–IV diagnosis of BN with no history of AN. They must have engaged in binge eating (as defined in DSM–IV) followed by vomiting at least once a day for 3 days of the week over at least a 3-month period. All subjects must be right-handed.

ED groups must have been REC for 12 months or more prior to entering the study. Status of recovery will be

defined as follows: a) No bingeing, purging, restrictive eating or other ED related behaviors in the preceding 12 months; b) Maintained a stable weight (± 3.0 kg) between 90% and 120% ABW[128] for at least 12 months; c) Regular menstrual cycles for the preceding 12 months; d) Have values within the normal range for beta–hydroxy–buteric–acid (BHBA), glucose, and insulin during the evaluation phase. Subjects will be studied during the first 10 days of the menstrual cycle (early follicular phase).

Exclusion Criteria for REC AN, REC AN-BN and REC BN Women**:** a) Met diagnosis of alcohol or drug abuse or dependence in the 3 months prior to the study. Alcohol or substance use within 30 days prior to PET studies as determined by self-report, clinical or psychiatric interviews, or urine toxicology screen; b) Current diagnosis of an Axis I major affective or anxiety disorder or suicidal thoughts, or presence of other psychopathology that might interfere with ability to participate in the study, (e.g., requiring inpatient hospitalization or medication); c) Organic brain syndromes, dementia, psychotic disorders, or mental retardation; d) Neurological or medical disorders such as seizure disorder, renal disease including pyelonephritis and chronic cystitis, impaired renal function including hyponatremia (less than 135 meq/l), hypokalemia, raised BUN (more than 15 mg/dl), raised creatinine (more than 1.5 mg/dl), diabetes, thyroid disease including hyperthyroidism and hypothyroidism, EKG indicative of electrolyte imbalance; e) BN subjects whose primary or only purging methods were the use of laxatives, diuretics or means other than vomiting; f) Use of psychoactive medication in the 3 months prior to the study; g) Pregnancy, lactation or lack of effective birth control during the 15 days before the scans. A pregnancy test will be conducted within 24 hours of the study; h) Tobacco use in the 3 months prior to the PET studies.

Criteria for CW**:** Healthy CW must not have any stigmata suggestive of an ED. They must have maintained an ABW between 90% and 120% since menarche. Their lab tests, medical and psychiatric histories, and physical and neurological examinations should indicate no current or past psychiatric (definitive Axis I disorder), medical or neurological illness. We recognize that these criteria will create a selection of healthier than average control subjects. However, it was deemed more important to avoid the potentially confounding effects of psychiatric symptomatology in the control group with regard to the biological measures for the current study. All pregnant and lactating women will be excluded.

11. RECRUITMENT

This is a five-year study in which we will recruit equal numbers of subjects each year. It is our experience from previous studies that it will be necessary to enroll an additional 20% of subjects due to drug failure, non-completion of PET studies, etc. Thus, we expect to enter 30 subjects to complete cells of 25 radioligand/PET studies per subject group. Overall, we will enter 120 subjects into this study in 5 years or 24 subjects per year.

Since at least 50 to 70% of individuals who have anorexia or bulimia eventually recover, we are confident that our recruitment goal can be achieved over the proposed 5-year period. All CW will be recruited from the greater San Diego region to minimize travel cost. However, based on our experience, approximately 75% of our REC subjects will come from outside southern California.

We will continue to employ recruitment strategies that were effective in our previous studies. These include: a) mailings to former patients of UCSD and the University of Pittsburgh ED treatment programs and other nationally recognized ED treatment programs directed by colleagues; b) mailings of IRB approved flyers to clinicians and treatment centers specialized in EDs, and to university health and counseling centers; c) placing advertisements and study announcements in ED related publications, press releases and conferences (e.g., NEDA, AED and EDRS); d) placing study advertisements on websites such as EDReferral and NEDA. We will also utilize the UCSD research websites which make available to professionals and the lay public alike our research publications and current findings; e) We may also contact participants who have been in our previous studies and who appear to be appropriate potential participants for this current study, provided they have given written permission to recontact them. There will be no cold-calling to any potential participant.

Subjects will contact our staff by either phone or email at which time we will ask initial screening questions which do not contain any identifiable information: age, weight, height, use of medications, handedness and presence of metal in the body. Our procedure is to establish if prospective study participants meet minimal criteria as described before proceeding to explain the protocol and obtain address to send out consent form and self-report questionnaires. If subjects are disqualified at this time their information will be destroyed. If preliminary requirements are met, this information is destroyed (page 1 of brief screening tool) and we would then proceed to obtain mailing information. This information is the same as would be asked if the subject was being set up for a clinical appointment. The only information retained will be name and address, and phone number. The protocol will then be explained to the subject and we will then mail a consent form and self-assessment questionnaires to the subject. If a subject declines to participate or does not meet the minimal required inclusion criteria as per brief screening tool, the information obtained will be destroyed. This information will also be destroyed after receiving a signed consent form back from a subject or in the case of no response after sending the consent information to a subject.

Recruitment will be accomplished by posting IRB approved flyers, website ads, craigslist, The San Diego Reader and ads in local and regional publications. Potential subjects can respond to these advertisements by phone or in writing. Many potential participants will live outside the immediate San Diego area; these potential research subjects would probably be unwilling to participate if they were required to come to UCSD to sign a written consent and undergo the initial screening. Also, because many subjects are excluded before completing the entire initial screening, requiring a trip to UCSD for a preliminary screening would be very time and cost intensive and therefore delay the completion of the study. Therefore, these subjects approaching us about participating in research will undergo a brief interview or telephone screening conducted by our research staff. This screening is designed to give a preliminary indication of whether or not the subject will be appropriate for this research study based upon current age, height, weight, and if the subject is currently on any medications. The preliminary screening tool is enclosed. It contains no name or identifier, (page 1). The only information retained will be name and address, (page 2). Written consents will then be obtained from the participant before any information is obtained for the in-depth screening process. If potential subjects appear to meet inclusion criteria (age, weight, height, and absence of medications) the research program will be explained to the subjects. All information is confidential and would not affect an individual's employability or reputation.

12. INFORMED CONSENT

Once a potential study participant expresses interest in our study and meets preliminary eligibility requirements, as determined by the brief phone screen, a written consent form will be sent to the subject either electronically or via mail. Consenting procedures will be HIPAA compliant. Upon receipt of written consent, recruitment contact will further explain by phone the details of the consent. The subject recruiter will present to study participants the objectives, procedures and a clear statement explaining risks and benefits involved in the study. Questions will be asked and elicited in order to ascertain that participants comprehend the study procedures as well as potential risks involved, prior to consenting to the study. All study related questions from study participants that a research staff is unable to address will be referred to one of the principal investigator, Drs. Kaye and Bailer, or other co-investigators. Once a participant signed the consent form, the participant is considered enrolled in the study.

Participants will sign a separate consent form if they are interested in the future genetic study. The same consenting procedure described above will be followed.

13. ALTERNATIVES TO STUDY PARTICIPATION

This is not a therapeutic study, and there are no alternatives.

14. POTENTIAL RISKS

Risks of Psychological and Cognitive Assessments. The main risks are that clinical interviews, cognitive testing and self-report questionnaires are time consuming. Material elicited during interviews may be upsetting to some study participants. Others may find cognitive tasks to be challenging or frustrating. These assessments, however, rarely pose psychological risks for study participants. It is our experience that REC ED subjects and CW will be willing to cooperate with such studies. These studies have not been psychologically traumatic to REC ED patients in the past. It should be noted that REC ED subjects are often motivated by altruistic feelings and are usually cooperative with studies that serve to develop a better understanding of EDs. We do not feel that these studies pose any significant psychological risk for the patients.

Risks of Venipuncture and Blood Sampling. There is minor discomfort and potentially significant psychological distress associated with venipuncture, if the procedure is not well-handled. There is a rare risk associated with the chance of infection or light-headedness or fainting due to blood withdrawal (less than 1 out of 100 people) and a more common risk (10 to 25 out of 100 people) of slight pain, bruising, bleeding or soreness associated with taking blood from a vein.

Risks of Catheterization. Insertion of a catheter for intravenous injection of the PET radiopharmaceutical is rarely associated with the chance of infection (less than 1 out of 100 people), but more commonly associated with slight pain or bruising at the puncture site (10-25 out of 100 people).

Risks of MRI. According to the FDA, there is currently no evidence that MRI with approved scanners of up to 4 Tesla signal strength are associated with adverse effects. However, there are three major sources of potential risks. First, the subject may experience discomfort being in the confined and sometimes noisy environment of the scanner. Second, the strong magnetic field will affect electronic, magnetic and metal devices that subjects may carry with them or that have been implanted in the their bodies. Third, if unprotected, hearing loss can occur with prolonged exposure to the noise generated during MRI imaging. In addition, subjects may temporarily experience dizziness and/or nausea if the individual moves rapidly through the magnetic field. There is also the potential for RF-induced local heating and for mild electric stimulation due to induced currents from rapidly switching gradients.

Risks of administering tracer dose of [^{11}C]DASB and [^{11}C]raclopride. Mass doses of radiotracers used in these studies are negligible. For these compounds, with affinities in the pico- to nano-molar range, the receptor occupancy is negligible, and we have not observed any pharmacological effects. However, as with any drug, the possibility of idiosyncratic reaction exists as discussed in the consent forms.

Risk of administering oral AMPH. The major somatic side effects of AMPH administration are cardiovascular (hypertension, palpitations, tachycardia, bradycardia, orthostasis, syncope). General effects such as sweating, feeling warm or cold, chest tightness, nausea, diarrhea, vomiting, muscle and abdominal cramping, blurred vision, and headaches, have been reported. There is a rare risk of permanent neurologic damage and death as a result of cardiac arrest or stroke. One of the largest cohort (n=81 schizophrenic and n=7 control subjects) of published safety data for 0.5 mg/kg oral AMPH is a report by Angrsit et al.[3] In this report, the greatest changes from baseline were observed over the first hour, and absolute readings peaked at the second hour, at which point mean BP indices in subjects who received AMPH were 31 mm Hg greater for SBP (systolic blood pressure), 11 mm Hg for DBP (diastolic blood pressure), and 18 mm Hg for MAP (mean arterial pressure). They also observed a mild but significant increase in heart rate (+ 10 beats per minute from baseline) after 3 hours in the healthy controls but not in subjects with schizophrenia. Several other groups have successfully used oral AMPH in the dose range of 0.43-0.5 mg/Kg in PET imaging studies, but they have not reported any adverse effects that required medical intervention.[49, 143, 193, 194] In addition, we have not experienced any adverse effects in our pilot study.[10]

Risks of Radiation Exposure: Participation in the PET imaging procedures involves exposure to small amounts of radiation. The EDE per single injection is 0.39 rems for [^{11}C]DASB and 0.25 rems for [^{11}C]raclopride. If the participants undergoes the maximum of 3 PET transmission scans, two [^{11}C]raclopride scans (10 mCi) and one [^{11}C]DASB scan (15 mCi), the combined Effective Dose Equivalent (EDE) radiation exposure will be equivalent to a whole body dose of 1.23 rem (12.3mSv) (24.64% of the annual whole body radiation exposure (5 rems) permitted to radiation workers by federal guidelines). This amount is approximately 7 times more than they would receive from the average person's annual background radiation exposure, which is approximately 160 mrem (1.6 mSv). This radiation dose is not expected to produce any harmful effects, although there is no known minimum level of radiation exposure considered to be totally free of the risk of causing genetic defects or cancer. The risk associated with the amount of radiation exposure participants received in this study is considered low and comparable to everyday risks and similar to that from many commonly performed diagnostic Nuclear Medicine Procedures. If participants are especially concerned with radiation exposure or have had a lot of x-rays already, we advise that they should discuss their concern with their doctor.

No PET studies will be performed on nursing, pregnant, or potentially pregnant women, as confirmed by serum pregnancy testing and a urine test. Adverse effects of the radiopharmaceuticals in the study have not been reported. However, the possibility exists for a rare reaction to any of the substances or procedures to which the subject is exposed. Insertion of catheters for intravenous injection of the PET radiopharmaceutical may be associated with slight pain or bruising at the puncture site.

Risk of Breach of Confidentiality. Breaches in confidentiality could impact future insurability, employability, or reproduction plans, or have a negative impact on family relationships, and/or result in paternity suits or stigmatization.

Risk of Alcohol Exposure: The production of the [^{11}C]raclopride compound contains up to 10% alcohol. This would amount to a total injected alcohol amount of 2mL of ethanol. Two mL distributed over 5000 mL whole blood or 2500 mL plasma would represent 2/5000 = 0.4 x 10-3 (Blood alcohol Concentration BAC 0.0004) or 2/2500 = 0.8 x 10-3 (BAC 0.0008) as maximum amount.

Risks of psychological assessments: The main risks are that clinical interviews, cognitive testing and self-report questionnaires are time consuming. Material elicited during interviews may be upsetting to some study participants. Others may find cognitive tasks to be challenging or frustrating. These assessments, however, rarely pose psychological risks for study participants.

MRI: The subject may experience discomfort being in the confined environment of the scanner.

Exposure to a high magnetic field. The only known hazard associated with exposure to a static high magnetic field is that the magnet exerts a strong force on ferromagnetic objects. For this reason, ferromagnetic objects are excluded from the vicinity of the magnet so that they will not become projectiles. In addition, each subject undergoes a standard screening procedure to determine whether they have any implanted materials that may pose a risk. If there is any doubt about the nature of any implanted material, the subject will not be scanned. Conventional MRI uses 1.5 Tesla (T) magnets. At the Keck Center for Functional MRI, the research systems for human use have field strengths of 3T. Imaging at these field strengths is not considered a significant risk according to FDA guidelines.

Heating from radiofrequency (RF) pulses. The RF pulses that are used for creating the MR signal deposit some energy in the body in the form of heat, but no ionizing radiation is used with MRI. For the same pulse

sequence, the RF power deposited is higher at higher magnetic field strengths. However, the pulse sequences we will use at 3T has relatively low power depositions. In the future pulse sequences with higher RF power depositions may be developed, but we will insure that the power deposited is always below the FDA guidelines.

Peripheral nerve stimulation from rapidly switched magnetic fields (dB/dt). Magnetic field gradients are switched on and off during imaging to encode the spatial distribution of the MR signal. Gradient switching rate depends on the gradient coil used, but does not depend on field strength. For this reason, the gradient switching rates will be similar to those for the 1.5 T scanners we have used in the past, and these rates will not exceed FDA recommendations. The FDA guideline states that a significant risk is involved only when "dB/dt sufficient to produce severe discomfort or painful stimulation" is used. All of the MRI studies performed in the past at 3T systems at UCSD are well below this threshold.

Acoustic noise. Acoustic noise is always an unwanted side effect of MR imaging. As currents are pulsed through the gradient coils within the magnetic field, the system acts like a loudspeaker, making a repetitive tapping sound. At the higher field strengths (3T) the acoustic noise is increased. In all our studies subjects will wear ear plugs to reduce the noise to a comfortable, safe level. The FDA guideline for a significant risk due to acoustic noise is "peak acoustic noise over 140 dB", and we will insure that the acoustic levels remain well below this value.

Acquisition of Eye Blink Rate. The main risks associated with acquisition of eye blink data are associated with time consumption. Participants may become bored or irritated having to sit still and hold a fixation on a computer screen for 5 minutes. However, acquisition of this data should cause minimal risk to the research participant.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

It is our experience that ED subjects and CW will be willing to cooperate with such studies. These studies have not been psychologically traumatic to ED patients in the past. It should be noted that ED subjects are often motivated by altruistic feelings and are usually cooperative with studies that serve to develop a better understanding understanding of EDs. We do not feel that these studies pose any significant psychological risk for the patients.

Risks of Psychological and Cognitive Assessments. While most study participants will find the clinical interviewing process to be a positive experience, some may become anxious or tearful because of the personal nature of the questions. Clinical interviewers will exercise caution and sensitivity and terminate the interview as necessary in order to protect the emotional well-being of the study participant. Similarly, study participants who experience frustration during cognitive assessments will be reassured that they are there to do their best and that it does not matter how well they score. They will be encouraged to continue but allowed to stop if continued distress is experienced. If the subject does not want to talk about any of her feelings during the study, she does not have to so, and she can tell the interviewer she does not want to talk about her feelings.

Risks of Venipuncture and Blood Sampling. We have a firm policy to only attempt venipuncture a maximum of twice on one occasion. In addition, we allow adequate time for the procedure, as necessitated by the individual subject. Very rarely will a subject be so anxious about venipuncture that these studies are not feasible. The taking of a blood sample may cause some discomfort. The CTRI nurse and the study staff will monitor the subjects' condition closely to minimize any discomfort.

MRI Related Risks. Typically, 3 Tesla scanners have been measured to produce noise between 122-131 db.[59] The potential risk of hearing loss associated with the noise generated during MRI is eliminated by using ear plugs (rated to reduce noise by 32 dB) and through the use of headphones (rated to reduce noise 30 dB).

Subjects will be strongly encouraged to move slowly when entering or exiting the magnetic field. The scanner bed has been designed to slowly move the subject so as to minimize the possibility of vertigo. RF-induced local heating is unlikely due to the software requirement that subject's weight is accurately recorded and used in determining the appropriate changes in gradients. Subjects are warned of the possibility of mild electric stimulation and are monitored throughout the session. Exclusions will be made for the following: cardiac pacemaker, metal fragments in eyes/skin/body (shrapnel), history of being a metal worker/welder, history of eye surgery/eyes washed out because of metal, aortic aneurysm clips, prosthesis, by-pass surgery/coronary artery clips, hearing aid, heart valve replacement, subjects with an IUD (birth control device), a shunt (ventricular or spinal), electrodes, metal plates/pins/screws/wires, or neuro/bio-stimulators (TENS unit), vision problems uncorrectable with lenses, claustrophobia, inability to lie still on one's back for approximately 30 minutes, prior neurosurgery, older tattoos with metal dyes, unwillingness to remove nose, ear, tongue or face jewelry, braces or permanent dental retainers, and women with a positive pregnancy test or currently lactating.

In addition, computers and the interfacing devices will be checked regularly for proper and safe operation. Similarly, study participants will be asked questions pertaining to fear of closed spaces during the screening stage. However, should the participant become claustrophobic during the study or for any reason be unable to endure remaining in the scanner, the study will be terminated and the participant will be removed from the scanner. In addition, a pregnancy test will be performed within 24 hours of the study. No studies will be performed on pregnant or potentially pregnant women. If there is a possibility of metal being present in the participant's body, the participant will be asked to undergo an x-ray study to rule out the presence of metal. The participant will be required to sign an additional consent form to undergo the x-ray study.

In order to minimize the risk of fear of closed spaces while in the MR scanner, patients will be extensively interviewed and informed about the nature of the task. If, at any point in during the scanning, the subject expresses increasing discomfort, the PI will immediately intervene and terminate the scanning procedure

Exposure to high magnetic fields: All subjects will be screened twice before they will be allowed to enter the MRI facilities. Any non-removable magnetic material will automatically exclude subjects. All staff is regularly trained in MRI safety procedures by the PI.

Heating from radiofrequency (RF) pulses: The MRI pulse sequences that will be used are discussed prior to usage by the MR physicists and the director of the Center for Magnetic Resonance Imaging (CMR) with respect to potential heating effects. The physicists at the CMR regularly test pulse sequences on phantom objects for heating effects.

Acoustic noise: Subjects will be instructed to wear earplugs or headsets that reduce the noise by about 20-40 dB. Subjects will be instructed to indicate if the noise is a source of physical or psychological discomfort. If this were to occur the experiment will be terminated immediately.

PET and Radiation related issues The use of [^{11}C] radioligands in this research proposal is considered to be generally safe and effective as approved by the University of California, San Diego Radioactive Drug Research Committee in accordance with Food and Drug Association regulation 21 CFR 361.1. No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing performed within 24 hours prior to each PET scanning session. The total Occupational Dose Limit / EDE for adults, as regulated by the US Nuclear Regulatory Commission is 5.0 rem per year. Thus, for the three studies the EDE is about 24.64% of the radiation dose (5 rem) permitted on an annual basis to radiation workers by federal regulations (21 Code of Federal Regulations (CFR) 361.1. If an X-ray study is required to rule out the presence of metallic fragments, the maximum radiation dose to the involved body area will be 0.3 rems (a unit of radiation dosage), with minimum exposure of the other areas of the body. In order to restrict the yearly cumulative exposure to

radiation as outlined in FDA 21.361.1, all subjects exposed to radiation in the work place are excluded, as well as subjects exposed to nuclear medicine procedures during the previous year, including research protocols. In case of idiosyncratic reactions to the radiotracers, a physician is present at each experiment and will be available at all times during the study and an emergency cart will be in close proximity.

Risk of Alcohol Exposure: The radiotracer that is injected will contain a very small amount of alcohol that is 1/1000 of what is allowed for driving a car in most states. This amount is minimal (Blood alcohol Concentration BAC 0.0004). The common legal limit for driving is a BAC of 0.8. In most cases the injected dose will be below. We do not expect that the subject will even recognize any effects from this amount of alcohol; however, we discourage subjects with a history of alcohol dependence from participating in order to avoid the risk of relapse.

Participant Monitoring on AMPH: Subjects with a history of hypertension or any heart disease will be excluded. Only subjects who are normotensive (100/70>BP<120/80) and have a HR above 55 bpm at screening, on the day of study before the 1st [11C]raclopride scan, as well as when measured 15 minutes after the 1st [11C]raclopride scan when subject will lay supine for 5 minutes in a quiet room and have BP and HR measured, and then stand for 1 minute when HR and BP will be repeated, will receive amphetamine. As amphetamine cross-reacts with street drugs such as cocaine, a rapid drug screen will be performed on the day of the scan. The dextroamphetamine will be administered to the subject by a physician familiar with the protocol (study physician, Dr. Ursula Bailer). A study physician will be available to monitor the subject until discharge. In order to monitor possible side effects of amphetamine including cardiovascular (hypertension, palpitations, tachycardia, bradycardia, orthostasis, syncope) as well as general effects such as sweating, feeling warm or cold, chest tightness, nausea, diarrhea, vomiting muscle and abdominal cramping, blurred vision and headaches, blood pressure and heart rate will be recorded every two minutes for the first 15 minutes after amphetamine administration, then every five minutes for 30 minutes, then every 15 minutes thereafter (up to at least 180 min) and then hourly until discharge. In addition, a participant will be asked to fill out an adverse event questionnaire after amphetamine ingestion in the following intervals: 10 min, 20 min, 30 min, 45 min, 60 min, 90 min, 120 min, 150 min and 180 min. If the patient has a SBP>190 or DBP>130 for greater than 5 minutes after ingestion of AMPH, then treatment with oral labetalol (200-400 mg) will be initiated. Sublingual nitroglycerin (150-600 mcg) will be administered if the patient complains of chest pain. All changes from a laying to a sitting, laying to a standing, or sitting to a standing position of the participants will be assisted by the staff on site. Participants will be requested to drink at least 1.5 liter of water between standardized breakfast and end of the 2nd scan. If the patient has psychomotor activation or behavioral symptoms suggestive of a hypomanic state, they will be treated with oral lorazepam 2 mg. If symptoms of nausea, diarrhea, muscle and abdominal cramping, or headaches occur and are persisting and becoming intolerable for the participant, we will provide respective medication (including antiemetic medication, spasmolytics and pain medication) based on the individual clinical severity and assessed by the PI and study physician, Dr. Ursula Bailer. All these medications will be stored at the UCSD NeuroPET center for emergency management. Following emergency treatment with these medications, the appropriate routine medical care will be initiated and provided. If any of the above side effects persist for more than 1 hour, despite the respective medication administered as outlined below, or intensify, the participant will be sent to the nearest Emergency Department. After a syncopal event the participant will be sent to the nearest Emergency Department immediately. Following the scans, the subject will receive: a) physical exam; b) vital signs measurement; c) mental status exam and d) EKG

Transfer Criteria: The subject will be transferred from the UCSD PET center to the UCSD Eating Disorder Treatment and Research Center once they meet the following criteria upon evaluation by a study physician: a blood pressure of less than 150/90 mm Hg; a heart rate of less than 110 BPM; no behavioral abnormalities.

Discharge Criteria: After a minimum of 6 hours of observation following drug ingestion, the subject will be medically discharged from the UCSD Eating Disorder Treatment and Research Center only if they meet the following criteria upon evaluation by a study physician: a) a blood pressure (BP) of less than 20 mm Hg above

their baseline reading for systolic and diastolic BP or less than 150/90 (whichever reading is lower); b) a heart rate of less than 20 BPM above their baseline or 100 (whichever reading is lower); c) no reported subjective effects from the AMPH; d) no adverse events to the AMPH; e) a normal physical exam; f) a normal mental status exam. Most of the REC AN and BN will travel to San Diego for this study. We will establish a policy that they are required to stay the night after the study and will be discharged to travel home the next day. For the subjects that live in San Diego, we will ask a relative to drive them home after being discharged from the study.

Confidentiality. To minimize possible risk of breach of confidentiality, each study participant will be assigned, upon entry into the study, a numeric code which will be used as the identifier for subsequent research related activities. All information collected, including self-report questionnaires, psychological assessments, and imaging data are identified solely by these numbers without any personal identifiers. Only strictly anonymous data will be entered into the database and used for statistical analysis. Access to the numbering system will be limited to the principal investigators, Drs. Bailer and Kaye, and their research staff involved in the study. Research files will be kept in locked quarters and made available only to qualified personnel for research purposes. No verbal or written information concerning a subject will be released to anyone without expressed written consent by the subject.
If blood samples are obtained for genetic sampling, all samples will be labeled by code numbers only and identifiable only by the PIs of this protocol, as knowledge of genetic research data could potentially impact future insurability, employability, or reproduction plans; or have a negative impact on family relationships; and/or result in shame or embarrassment.

Others. Unanticipated findings of potential medical significance uncovered during the course of the study, including a positive pregnancy test or an abnormal MRI, will be reported to the PIs. The study participant will be informed of the test result and then referred to her physician. If the participant does not have a regular physician, an appropriate referral will be made. If the participant does not have a regular physician, an appropriate referral will be made. In the unlikely event that a subject is injured during the study, she will receive care from the University of California as needed to treat the injury.

16. PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT

To minimize possible risk of breach of confidentiality, each study participant will be assigned, upon entry into the study, a numeric code which will be used as the identifier for subsequent research related activities. All information collected, including self-report questionnaires, psychological assessments and imaging data are identified solely by these numbers without any personal identifiers. Only strictly anonymous data will be entered into the database and used for statistical analysis. Access to the numbering system will be limited to the principal investigator, Drs. Kaye and Bailer, and the research staff involved in the study. Research files will be kept in locked quarters and made available only to qualified personnel for research purposes. No verbal or written information concerning a subject will be released to anyone without expressed written consent by the subject.
A data and safety monitoring plan will be implemented with close supervision by the principal investigators over all research-related activities. Drs. Bailer and Kaye will meet weekly with their research staff to review recruitment issues, data collection and entry and assess the adequacy of procedures to ensure subject privacy and confidentiality. More specifically, the data safety and monitoring plan will involve 1) evaluation of the progress of the research study, e.g., assessing data quality and timeliness of participant recruitment, accrual and retention; 2) review of the outcome and adverse event data to determine whether there is any change to the anticipated benefit-to-risk ratio of study participation and determination of whether the study should continue as originally designed, be changed or terminated; 3) assessment of external factors or relevant information that may have an impact on the ethics of the research or safety of study participants; and 4) review of study procedures designed to protect the privacy of the research subjects and the confidentiality of their research data. In addition, we will use a Data Safety Monitoring Board consisting entirely of personnel not associated with the

study, provided by the UCSD's CTRI. In the event of an adverse event, our research staff will immediately communicate with Drs. Bailer and Kaye, as well as the IRB, in compliance with IRB policy for reporting adverse events. All fatal or immediately life-threatening events as well as unanticipated problems (UPRs) will be reported in writing to the IRB as soon as possible and no later than 10 working days after discovery and in compliance with UCSD Human Research Protection Program Institutional Review Board Standard Operating Policies and Procedures (Section 3.13). The IRB will determine appropriate actions for mitigating unexpected problems. UPRs involving risks to human subjects or others will be promptly reported to OHRP, FDA, and the appropriate University officials. In addition, adverse events will be reported annually as a part of the Continuing Review in compliance with UCSD Human Research Protection Program Institutional Review Board Standard Operating Policies and Procedures (Section 3.11). If, during the course of the study, changes or modifications to the study protocol become necessary to ensure continued confidentiality measures, or to ensure the level of risk associated with this study, the IRB will be notified immediately.

17. POTENTIAL BENEFITS

Subjects participating in this study will benefit from the screening procedures that include a careful examination of the patient's psychiatric condition. Subjects will be paid for their participation since subjects will not accrue any other direct benefit for their participation in this study. Both experimental and control subjects will receive remuneration for their participation in these studies. Many subjects work or attend school, so that participation in this study will mean that they will have to take time off from these responsibilities. This payment is to serve as an incentive for subject participation and to reimburse travel, lost wages, and expenses (e.g., parking). Although the individual subjects may not receive any benefit beyond financial remuneration, the data generated by this project could significantly enhance our knowledge about the risk factors for EDs, and pave the way for new clinical treatments for these disorders. Study participants may also gain satisfaction from the knowledge that they are contributing to a better understanding of brain function and potentially to the etiology of a psychiatric illness. There is little in the way of proven treatments for ED, particularly AN. Consequently, this is a costly and often chronic disorder for many. A better understanding of the pathophysiology of AN and BN is likely to lead to better therapies.

18. RISK/BENEFIT RATIO

The information gathered in this study will contribute to understanding 5-HT and DA neuronal circuit function in REC ED subjects. This will help identify disease specific disturbances and hopefully lead to identify more effective pharmacologic treatment for EDs. In addition this study seeks to determine correlations of behavioral state and trait variables with 5-HTT and DA receptor activity. This will be helpful for both the study of ED pathology as well as the study of normal behavior-biology interrelationships. Eating disorders such as anorexia and bulimia nervosa are often chronic with high morbidity and mortality, and individuals and families incur enormous health care expenses. We strongly believe that the potential benefits of understanding of the pathology of ED outweigh the potential risks.

19. EXPENSE TO PARTICIPANT

There will be no fees or charges to the subject for any of the medical procedures or for the PET or MRI or evaluations required for the study, questionnaires or assessments.

20. COMPENSATION FOR PARTICIPATION

Subjects will be compensated $375.00 for participation in each of the [^{11}C]DASB PET scan and the 1st [^{11}C]raclopride scan, for a total of $1250.00 if they completed also the 2nd [^{11}C]raclopride scan after ingestion of amphetamine. Consented participants who do not meet all criteria will be paid $10 for completing just the written assessments, and $25.00 for completing the preliminary subjective assessments and physician interview. If a participant is ruled ineligible after completing the entire screening process with interviews, assessments, physical, labs and EKG, they will be compensated $50.00 for their time.

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

Walter H. Kaye, M. D., Professor of Psychiatry, is Director of the Eating Disorders Program at University of California, San Diego. These studies are a continuation of research protocols devised and carried out by Dr. Kaye on eating disorder patients since 1978. Dr. Kaye has published more than 300 papers on eating disorders and is board certified in psychiatry and board eligible in neurology.

Ursula F. Bailer, M.D., Assistant Clinical Professor, Department of Psychiatry, University of California San Diego, and Associate Professor of Psychiatry at the Medical University of Vienna, Department of Psychiatry and Psychotherapy, and Director of the Outpatient Clinic for Eating Disorders and Eating Disorder Research Program at the Medical University of Vienna, Austria. Dr. Bailer has extensive experience in PET analysis and modeling, including the aforementioned radiologands since almost ten years. Furthermore, Dr. Bailer has over 14 years of extensive experience in diagnosis, treatment and research of eating disorders in adolescents and adults. She is author on more than 50 scientific peer reviewed publications and 10 books /book chapters.

Julie Price, Ph.D. is a Professor of Radiology, Director, PET Pharmacokinetic Modeling, at the Athinoula A. Martinos Center for Biomedical Imaging at Massachusetts General Hospital and Member of the Faculty of Radiology, Harvard Medical School. Dr. Price has extensive experience in integrated approaches to the analyses of multiple intra-subject neuroimaging measures (e.g. blood flow, neuroreceptor binding) in studies of medical illness, neuropsychiatric disorders, and aging. She is an expert in PET modeling procedures for several radiotracers. Dr. Price has collaborated with Drs. Bailer and Kaye on PET studies in anorexia and bulimia nervosa at the University of Pittsburgh, and closely worked with Dr. Bailer on data analysis and interpretation of data for several PET projects. Dr. Price will provide expertise with regard to PET modeling, data analysis and interpretation.

Brian Lopresti, MSNE, is Instructor of Radiology. Mr. Lopresti is a nuclear engineer with more than 20 years of experience spanning PET radiopharmaceutical discovery and development, medical physics, nuclear instrumentation and radiation detection methods, tracer kinetic analyses, and quantitative PET data analysis. Mr. Lopresti's research efforts have focused on the development and analysis of PET radiotracers and methods for assessing neurodegenerative disease pathology, neuroinflammation, and neurotransmitter dysfunction in human subjects, having authored or co-authored more than 60 publications in this area. Since 2003, Mr. Lopresti has been responsible for directing preclinical PET research efforts at the University of Pittsburgh PET Facility, for which the use of non-human primate models of CNS diseases is an emphasis. More recently. Mr. Lopresti was been appointed as Head of PET Methodology and Data Analysis. In this role, Mr. Lopresti has oversight of all data analysis activities relating to PET image data collected at the University of Pittsburgh PET Facility.

Monte S. Buchsbaum, M.D., Distinguished Professor of Psychiatry and Radiology in March 2009. He heads the UCSD NeuroPET Center and leads an effort in developing an expanded research effort with positron emission tomography. Since 1980, the primary focus of Dr. Buchsbaum's research has been brain imaging, combining positron emission tomography, computerized EEG and evoked potential brain mapping, and later magnetic resonance imaging and diffusion tensor imaging. While in the Intramural Research Program at the National Institute of Mental Health, Dr. Buchsbaum published some of the first PET studies in psychiatry. In 1982, Dr. Buchsbaum left the NIMH to head the Brain Imaging Center at the University of California, Irvine (UCI) and carried out studies on the prefrontal cortex in schizophrenia using FDG-PET and EEG. In 1992, Dr. Buchsbaum moved to New York to head the Neuroscience PET laboratory, focusing on pharmacological response and PET-FDG. In 1998 he published the first report on diffusion tensor imaging in schizophrenia, extending his work on potential deficits in the prefrontal cortex in schizophrenia. He is Editor of Psychiatry Research and Co-Editor of Psychiatry Research: Neuroimaging. He has published over 485 research reports in scientific journals, the majority on positron emission tomography. He is included in the Institute of Scientific Information data base as

one of the most highly-cited scientists (ISIHighlyCited.com).

Carl Hoh, M.D. Associate Professor of Radiology, Division Chief of Nuclear Medicine at the University of California, San Diego. The quantitative PET research protocols have been a performed by Dr. Hoh since his training in Nuclear Medicine & PET at UCLA from 1989. Dr. Hoh has multiple publications on quantitative PET imaging.

Kishore Kumar Kotta, Ph.D., Staff Research Associate IV. Department of Radiology, UCSD Center for Molecular Imaging, University of California, San Diego, Ca
Kishore K. Kotta obtained his Ph.D., in Synthetic Organic Chemistry from Department of Chemistry, University of Akron, OH and has more than 15 years of research experience in the areas of Synthetic Organic Chemistry, Medicinal Chemistry, Drug Delivery, and Radio Chemistry. His contributions to the field of chemistry resulted in many publications in various international journals as well as scientific meetings. He has been working in Radiochemistry lab at University of California, San Diego since 2010 developing new PET radiopharmaceuticals for clinical studies.

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24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

Biological Materials will be kept under locked conditions at UCSD Eating Disorder Treatment and Research Center with Dr. Kaye for an indefinite time where they will be submitted for analysis with DNA and lymphocyte extraction. No biological materials transfer agreement is necessary.

25. INVESTIGATIONAL DRUG FACT SHEET AND IND/IDE HOLDER

Not Applicable

26. IMPACT ON STAFF

Nursing staff will be utilized in accordance with CTRI policies.

27. CONFLICT OF INTEREST

Not Applicable

28. SUPPLEMENTAL INSTRUCTIONS FOR CANCER-RELATED STUDIES

Not Applicable

29. OTHER APPROVALS/REGULATED MATERIALS

Not applicable

30. PROCEDURES FOR SURROGATE CONSENT AND/OR DECISIONAL CAPACITY ASSESSMENT

Not Applicable

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