Title: Toward understanding dopamine receptor contributions to prediction error and reversal learning in anorexia nervosa

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Study Protocol Date: 04/12/2021

UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN

Instructions for completing the Research Plan are available on the HRPP website.
The headings on this set of instructions correspond to the headings of the Research Plan.
General Instructions: Enter a response for all topic headings.
Enter "Not Applicable" rather than leaving an item blank if the item does not apply to this project.

Date: 04/12/2021

1. PROJECT TITLE

Toward understanding dopamine receptor contributions to prediction error and reversal learning in anorexia nervosa

2. PRINCIPAL INVESTIGATOR

Guido K.W. Frank, MD

3. FACILITIES

1. UC San Diego Health Eating Disorders Center for Treatment and Research
2. Altman Clinical Trials Research Institute (ACTRI)
3. Center for Advanced Laboratory Medicine (CALM)
3. Sharp and Children's MRI Center
4. Medical Care San Diego
5. LabCorp

4. ESTIMATED DURATION OF THE STUDY

3 years enrollment, 2 years data analysis (5 years total)

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

Anorexia nervosa (AN) is an eating disorder associated with intense fear of weight gain, food refusal, and severe weight loss. AN has the highest mortality rate among the psychiatric disorders; however, little is known about biomarkers, and no medication has been approved for AN. Brain reward pathways have a direct impact on the drive to eat, and a variety of neuroimaging studies have suggested altered reward processing in AN. The neurotransmitter dopamine has a central role in the reward circuitry to drive food approach, and the dynamic interplay between dopamine receptor response and food restriction could have implications for the pathophysiology of AN. In this application, we will develop a study protocol and gather pilot data to identify whether specific dopamine D2 challenge drugs can modulate PE response and reversal learning in AN. In this randomized placebo controlled trial we will apply a dopamine D2 receptor agonist, D2 receptor antagonist and placebo to healthy controls and individuals with AN to test their effects on brain reward processing and reversal learning. We expect that those challenge drugs will lead to opposite brain and behavioral response and directly support the involvement of those receptors in brain function in AN. This research will be important to develop neurotransmitter specific pharmacological treatments for AN.

6. SPECIFIC AIMS

Aim 1. To establish in AN feasibility and acceptability of a study design to understand the contributions of the selective DA D2 receptor agonist bromocriptine on PE and reversal learning.
Hypothesis 1.a. The DA D2 receptor agonist bromocriptine (1.25mg) will enhance fMRI PE response during a taste-reward PE paradigm in AN in ventral striatum and insula compared to placebo.

Hypothesis 1.b. The DA D2 receptor agonist bromocriptine (1.25mg) will enhance reversal learning and striatum brain response in AN during a monetary reversal learning task compared to placebo during fMRI.

Aim 2. To establish in AN feasibility and acceptability of a study design to understand the contributions of the selective DA D2/D3 receptor antagonist amisulpride on PE and reversal learning.
Hypothesis 1.a. The DA D2 receptor antagonist amisulpride (400mg) will reduce fMRI PE response in the insula and ventral striatum compared to placebo.
Hypothesis 1.b. The DA D2 receptor antagonist amisulpride (400mg) will reduce reversal learning and striatum brain response in AN during a monetary reversal learning task compared to placebo during fMRI.

Aim 3. To gather pilot data how fMRI brain response to the DA D2 receptor agonist bromocriptine or antagonist amisulpride differs between individuals with AN and healthy control women.
Hypothesis 3. We expect we will find an indication that bromocriptine will enhance PE (ventral striatum and insula) and reversal learning (frontal cortex) in AN more compared to the control group, while amisulpride causes the opposite.

7. BACKGROUND AND SIGNIFICANCE

Anorexia nervosa (AN) is an eating disorder associated with intense fear of weight gain, food refusal, and severe weight loss (American Psychiatric Association, 2013). AN has the highest mortality rate among the psychiatric disorders (Sullivan, 1995; Arcelus et al., 2011), and the relapse rate is high with up to 52% (Khalsa et al., 2017; Berends et al., 2018). Many individuals only partially recover, and treatment options, especially for the psychological components of the illness, are not very effective (Berends et al., 2018; Murray et al., 2018). Little is known about biomarkers for AN, and no medication has been approved for AN (Simansky, 2005).

Brain reward pathways have a direct impact on the drive to eat, and a variety of neuroimaging studies have suggested altered reward processing in AN (Uher et al., 2004; Fladung et al., 2010; Kaye et al., 2013b; Frank, 2015; Monteleone et al., 2018). The neurotransmitter dopamine (DA) has a central role in the reward circuitry to drive food approach (Kelley et al., 2005), and the dynamic interplay between DA receptor response and food restriction (or overconsumption) could have implications for the pathophysiology of AN (Kelley, 2004; Johnson and Kenny, 2010; Carr, 2011). Recent clinical studies indicate that the DA D2 receptor partial agonist aripiprazole may be beneficial in the treatment of AN (Trunko et al., 2011; Frank et al., 2017). However, aripiprazole also acts on serotonin and other neurotransmitter receptors and whether aripiprazole's DA D2 receptor agonism is indeed beneficial for AN treatment is unknown (Frank, 2014).

DA-related brain function in humans has been studied indirectly using functional magnetic resonance brain imaging (fMRI) and tasks that deliver reward stimuli unexpectedly, eliciting the so-called prediction error (PE) response (Schultz, 2002; O'Doherty et al., 2003). Research in adult AN showed elevated PE response in insula and striatum (Cowdrey et al., 2011; Frank et al., 2012). Studies in adolescents using monetary or taste reward stimuli also resulted in heightened PE responses in those regions, and orbitofrontal PE predicted weight gain in treatment (DeGuzman et al., 2017; Frank et al., 2018a). DA and PE response have also been associated with altered reversal learning (Izquierdo et al., 2017), which has important treatment implication for AN as reversal learning is impaired in the disorder, and modulation of the DA system could improve treatment (Wildes et al., 2014; Allen et al., 2017; Foerde and Steinglass, 2017). Thus, the DA associated PE response promises to be an important biological marker for AN.

It has been suggested that phasic DA signals encode unexpected rewards and are associated with behavioral activation through DA D1 receptors (Maia and Frank, 2011). On the other hand, DA D2 receptors may code the dip in tonic DA neuron activity when stimuli are unexpectedly omitted, and may also be involved in avoidance learning and behavior inhibition (Maia and Frank, 2011). Those receptor dynamics are still being investigated and have not been studied in AN (Soares-Cunha et al., 2016).

In this study, we would like to use the exploratory/developmental R21 mechanism to develop a study protocol to gather pilot data to identify whether specific DA D2 challenge drugs are tolerated agents in the AN

population and whether they can modulate PE response and reversal learning in AN. This research will be important to develop neurotransmitter specific pharmacological treatments for AN. Studying also the DA D1 receptor would go beyond the financial limits of this funding mechanism but will be the target of future studies.

8. PROGRESS REPORT

Not applicable

9. RESEARCH DESIGN AND METHODS

Overview of Study Procedures:

In this randomized, placebo controlled trial subjects will complete a battery of self-assessments, a diagnostic assessment, and a pre-scan visit in order to determine eligibility. After eligibility is confirmed, subjects will be randomized to one of the six study arms that will determine the order in which the study medication is administered the morning of the MRI scans. Subjects will complete 3 MRI scans spaced approximately 3 days apart. Functional MRI will be performed and brain response will be measured while subjects complete a taste reward task and a reversal learning task. Details of the outcome measures and study procedures are listed below:

Outcome Measure(s):

Prior to completing the fMRI scans, each subject will complete the Diagnostic interviews and Self-Assessments listed below:

	Name	Abbreviation	Area assessed	Minutes
Diagnostic Interviews				
	Structured Clinical Interview for DSM-5 Diagnoses	SCID 5	Half structured diagnostic interview for Axis I Disorders	45-90
Self-assessments (Questionnaire Packet)				
	Spielberger State-Trait Anxiety Scale-Version Y	STAI-Y	State and trait anxiety	10
	Beck Depression Inventory	BDI-II	Assess the cognitive, behavioral, affective, and somatic symptoms associated with depression.	5
	Eating Expectancy Inventory	EEI	Measures cognitive expectations for eating	5
	Food Cravings Questionnaire - Trait	FCQ-T	Measures intensity of state and trait food cravings	10
	Intolerance of Uncertainty Scale	IUS	Measures intolerance of uncertainty	5
	Eating Disorder Examination	EDE-Q	Measures restraint and eating, weight and shape concern	15

	Temporal Experience of Pleasure Scale	TEPS	Measures anticipatory and consummatory experiences of pleasure for food and nonfood items	5
	Eating Disorders Inventory -3	EDI-3	Core ED symptoms	15
	Temperament and Character Inventory	TCI	Personality and temperament	30
	Sensitivity to Punishment and Sensitivity to Reward Questionnaire	SPSRQ	Reward and Punishment Sensitivity	5
	Beck Cognitive Insight Scale	BCIS	Subject beliefs and degree of confidence in beliefs	5
	Schedule for Assessment of Insight – Modification for Eating Disorders	SAI-ED	Insight into eating disorder symptoms	3
	Behavioral Inhibition Scale/Behavioral Activation Scale	BIS/BAS	Reward and Punishment Sensitivity	5
	Parental Bonding Instrument	PBI	Parental bonding	5
	Adverse Childhood Effects Questionnaire	ACEs	Childhood trauma	5
Scan Day Assessments				
	Food Cravings Questionnaire - State	FCQ-S	Measures intensity of state and trait food cravings	5
	Hedonic Taste Preference Scale for Sweet Stimuli	HTP S	Sweet taste pleasantness & sweetness ratings	5
	Taste Labeled Magnitude Scale	LMS	Taste sensation ratings	2
	Post Scan Taste Ratings		Scan solutions taste pleasantness & sweetness ratings	2
	Post MRI Questionnaire		Questions about pre-scan behaviors & problems during scan	5
	The Positive and Negative Affect Schedule	PAN AS	Measures current mood states, completed Pre-Scan, Post Scan and Post Meal	5
	Eating Expectancy Inventory	EEI	Measures cognitive expectations for eating	5
	Beck Depression Inventory	BDI-II	Assess the cognitive, behavioral, affective, and somatic symptoms associated with depression.	5
	Eating Disorder Examination	EDE-Q	Measures restraint and eating, weight and shape concern	15
	Temporal Experience of Pleasure Scale	TEPS	Measures anticipatory and consummatory experiences of pleasure for food and nonfood items	5

In addition to completing these measures, subjects will complete 3 fMRI scans. While in the MRI scanner, subjects will complete a Taste Reward Task and a Reversal Learning Task (described below).

Study Procedures Chart (Subjects from Community)

	Initial Phone Screen	Detailed Eligibility Screen	Screening Visit	Pre-Scan Visit	Study Visit 1	Study Visit 2	Study Visit 3
Initial Screening	**X**						
Informed Consent (through mail)		**X**					
Questionnaire Packet		**X**					
Biological Screening Form		**X**					
In person Consent and Interview with PI			**X**				
Diagnostic Interview			**X**				
Swallow Test			**X**				
Blood Tests & EKG				**X**			
Pregnancy Test					**X**		
Taste Test					**X**	**X**	**X**
MRI Scan					**X**	**X**	**X**
Scan Questionnaires					**X**	**X**	**X**

Study Procedures Chart (Subjects from UC San Diego Health Eating Disorders Center for Treatment and Research)

	Consent Visit	Screening Visit	Pre-Scan Visit	Study Visit 1	Study Visit 2	Study Visit 3
Initial Screening	**X**					

Informed Consent	**X**					
Questionnaire Packet		**X**				
Consent and Interview with PI		**X**				
Diagnostic Interview		**X**				
Swallow Test		**X**				
Blood Tests & EKG			**X**			
Pregnancy Test				**X**		
Taste Test				**X**	**X**	**X**
MRI Scan				**X**	**X**	**X**
Scan Questionnaires				**X**	**X**	**X**

TIMELINE

<u>Subjects from the Community</u>

Screening Visit
Location: PI office at UC San Diego Health Eating Disorders Center for Treatment and Research (4510 Executive Dr, Suite 330)

Subjects who meet eligibility criteria based on the results of the questionnaires and phone bioscreen will be invited to the in-person interview with the PI at the UC San Diego Health Eating Disorders Center for Treatment and Research. At the start of the study visit, the PI or research coordinator will review the consent form with the subject, will answer any questions about the study and will assess comprehension of the study procedures, benefits, and risks. Subjects will then sign a new consent form and a signed and dated copy of the completed consent form will be given to the subject.

Dr. Frank will meet with each subject to complete the diagnostic assessment (SCID-5) and comprehensive medical interview with the subject to determine study eligibility.

After eligibility is confirmed, the subject will complete a swallow test where she will be given an empty pill capsule and will be asked to swallow to make sure she will not have any issues with the size of the medication capsules. If the subject cannot swallow this size capsule or feels she will not be able to, she may be withdrawn from the study at that point.

The subject will be scheduled for the Pre-Scan Visit and 3 MRI scans. The MRI scans will be scheduled 2 to 4 days apart (3 days apart is ideal).

Pre-Scan Visit
Location: Altman Clinical Trials Research Center (9452 Medical Center Dr)

Subjects will complete a pre-scan visit at ACTRI to complete a blood draw and electrocardiogram (EKG). This visit will occur no more than 1 week before Study Visit 1 is scheduled. At this visit ACTRI research nursing staff (certified phlebotomist) will complete the blood draw (about 2 tablespoons blood). An EKG will be performed, and results of the EKG and blood tests will be sent to the PI for review. The following tests will be processed through the Center for Advanced Laboratory Medicine (CALM): Creatinine; BUN; electrolytes; serum pregnancy test. If the PI feels the results are out of range he may withdraw the subject from the study.

Alternatively, subjects may complete the Electrocardiogram (EKG) at Medical Care San Diego (7634 Girard Ave Ste A, La Jolla) and the labs (Creatinine, BUN, electrolytes and serum pregnancy test) at either the Medical Care San Diego (7634 Girard Ave Ste A, La Jolla) or a LabCorp location close to where the subject resides. Subjects who use these facilities to complete the EKG and labs will provide the PI and his staff with the test results. Subjects will need to complete these tests no more than one week prior to the first scheduled MRI scan. The research study will pay these facilities directly on the subject's behalf with the self-pay option. Once the PI receives the test results, if he feels the results are out of range he may withdraw the subject from the study.

Study Visit 1
Location: Sharp and Children's MRI Center (7910 Frost St)

The morning of the imaging procedures the subject will go to the Sharp and Children's MRI Center for the scanning procedures. The medication will need to be administered 3 hours before the start of the scan.

When the subject arrives at the MRI Center, subjects will complete a urine pregnancy test. After the pregnancy test (test must be negative), the research assistant will take the subject's blood pressure and measure her heart rate and administer the study medication. The subject will complete the Taste Labeled Magnitude Scale, Hedonic Taste Preference Scale, Food Cravings Questionnaire (State), Eating Disorders Examination (EDE-Q), Temporal Experience of Pleasure Scale (TEPS), Eating Expectancy Inventory (EEI), Beck Depression Inventory (BDI-II), The Positive and Negative Affect Schedule (PANAS), and eat a standardized breakfast.

The research staff will then review study procedures and fMRI tasks.

Subjects will then complete the fMRI and structural MRI (details of tasks outlined below).

After the scan, the research assistant will take the subject's blood pressure and heart rate and will have the subject complete the Post Scan Pleasantness Ratings and the Post MRI Questionnaire.

The total length of the study visit will be approximately 4 hours (3 hours pre-scan, 1 hour scan).

Study Visit 2
Location: Sharp and Children's MRI Center (7910 Frost St)

The morning of the imaging procedures the subject will go to the Sharp and Children's MRI Center for the scanning procedures. The medication will need to be administered 3 hours before the start of the scan.

When the subject arrives at the MRI Center, the research assistant will take the subject's blood pressure and measure her heart rate and administer the study medication. The subject will complete the Taste Labeled Magnitude Scale, Hedonic Taste Preference Scale, Food Cravings Questionnaire (State), Eating Disorders Examination (EDE-Q), Temporal Experience of Pleasure Scale (TEPS), Eating Expectancy Inventory (EEI), Beck Depression Inventory (BDI-II), The Positive and Negative Affect Schedule (PANAS), and eat a standardized breakfast.

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The total length of the study visit will be approximately 4 hours (3 hours pre-scan, 1 hour scan).

Study Visit 3
Location: Sharp and Children's MRI Center (7910 Frost St)

The morning of the imaging procedures the subject will go to the Sharp and Children's MRI Center for the scanning procedures. The medication will need to be administered 3 hours before the start of the scan.

When the subject arrives at the MRI Center, the research assistant will take the subject's blood pressure and measure her heart rate and administer the study medication. The subject will complete the Taste Labeled Magnitude Scale, Hedonic Taste Preference Scale, Food Cravings Questionnaire (State), Eating Disorders Examination (EDE-Q), Temporal Experience of Pleasure Scale (TEPS), Eating Expectancy Inventory (EEI), Beck Depression Inventory (BDI-II), The Positive and Negative Affect Schedule (PANAS), and eat a standardized breakfast.

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<u>Subjects from UC San Diego Health Eating Disorders Center for Treatment and Research</u>

Consent Visit (Subjects from UC San Diego Health Eating Disorders Center for Treatment and Research)

Location: UC San Diego Health Eating Disorders Center for Treatment and Research (4510 Executive Drive, Suite 315)

Subjects from these programs who are interested in the study will be scheduled to meet with a research staff member. Research staff and the PI will screen EPIC charts for potential subjects from UC San Diego Health Eating Disorders Center for Treatment and Research to determine initial eligibility. Prior to approaching the patient, Dr. Frank and/or his staff will obtain permission from the patient's individual therapist prior to making contact with the patient to describe the research opportunity.

The research staff will meet with each subject at UC San Diego Health Eating Disorders Center for Treatment and Research and go over initial eligibility. The research staff will review the consent form and study procedures with each subject, assess comprehension of study procedures, benefits and risks, and answer questions about the study.

A signed and dated copy of the completed consent form will be given to the subject.

Dr. Frank or a PhD level clinician will meet with each subject to complete the diagnostic assessment (SCID-5) and comprehensive medical interview with the subject to determine study eligibility.

After eligibility is confirmed, the subject will complete a swallow test where she will be given an empty pill capsule and will be asked to swallow to make sure she will not have any issues with the size of the medication capsules. If the subject cannot swallow this size capsule or feels she will not be able to, she may be withdrawn from the study at that point.

The subject will be scheduled for the Pre-Scan Visit and 3 MRI scans. The MRI scans will be scheduled 2 to 4 days apart (3 days apart is ideal).

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Alternatively, subjects may complete the Electrocardiogram (EKG) at Medical Care San Diego (7634 Girard Ave Ste A, La Jolla) and the labs (Creatinine, BUN, electrolytes and serum pregnancy test) at either the Medical Care San Diego (7634 Girard Ave Ste A, La Jolla) or a LabCorp location close to where the subject resides. Subjects who use these facilities to complete the EKG and labs will provide the PI and his staff with the test results. Subjects will need to complete these tests no more than one week prior to the first scheduled MRI scan. The research study will pay these facilities directly on the subject's behalf with the self-pay option. Once the PI receives the test results, if he feels the results are out of range he may withdraw the subject from the study.

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The research staff will then review study procedures and fMRI tasks.

Subjects will then complete the fMRI and structural MRI (details of tasks outlined below).

After the scan, the research assistant will take the subject's blood pressure and heart rate and will have the subject complete the Post Scan Pleasantness Ratings and the Post MRI Questionnaire.

The total length of the study visit will be approximately 4 hours (3 hours pre-scan, 1 hour scan).

Study Visit 2
Location: Sharp and Children's MRI Center (7910 Frost St)

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When the subject arrives at the MRI Center, the research assistant will take the subject's blood pressure and measure her heart rate and administer the study medication. The subject will complete the Taste Labeled Magnitude Scale, Hedonic Taste Preference Scale, Food Cravings Questionnaire (State), Eating Disorders Examination (EDE-Q), Temporal Experience of Pleasure Scale (TEPS), Eating Expectancy Inventory (EEI), Beck Depression Inventory (BDI-II), The Positive and Negative Affect Schedule (PANAS), and eat a standardized breakfast.

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Subjects will then complete the fMRI and structural MRI (details of tasks outlined below).

After the scan, the research assistant will take the subject's blood pressure and heart rate and will have the subject complete the Post Scan Pleasantness Ratings and the Post MRI Questionnaire.

The total length of the study visit will be approximately 4 hours (3 hours pre-scan, 1 hour scan).

fMRI Study Procedures

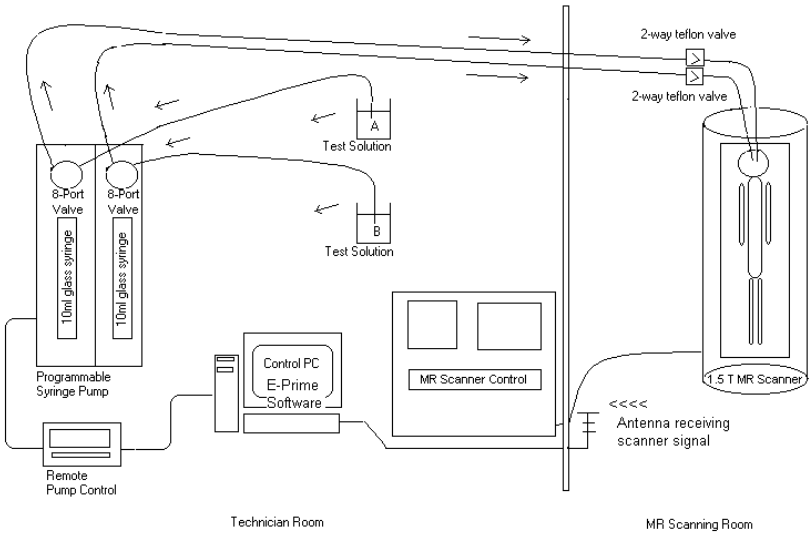
Location: Sharp and Children's MRI Center (7910 Frost St)

Experimental arrangements before experiment Procedure: All subjects will come to the Sharp & Children's MRI Center between 6 and 7 am (or 3 hours prior to the start of the scan). Subjects will have a urine pregnancy test (Study Visit 1 only), and all will receive a standardized breakfast and complete scan day questionnaires.

Taste fMRI – Expected and Unexpected sweet stimulus: In this study, subjects will get small samples (1.0 cc) of fluid that will consist of Sucrose or neutral solution (artificial saliva). These fluid samples will be administered through food grade polyethylene plastic tubes (1/8 inches inner, 3/16 inches outer diameter, FDA/USDA approved, Cole-Parmer, Vernon Hill, IL) that subjects hold in their mouth. In addition, the subjects will be trained to perform one tongue motion after each application of taste stimulant in order to wash the taste stimuli around the mouth and stimulate taste buds. The fluid will be released, at intervals, by a pump mechanism. Because of the small volume of fluid, there is no danger of aspiration. The subjects will be able to swallow the small amounts of solutions after application. Subjects that are not capable of carrying out these required procedures will be excluded from the study.

Syringe Pump Assembly: Fluid challenges will be delivered to the subject by an apparatus (Figure 3.1.) that will use a semi-automatic and programmable customized syringe pump (J-Kem Scientific, St. Louis, MO). The syringe pump will pump samples from 5 reservoirs through FDA approved food grade teflon tubes (Cole-Parmer, Vernon Hills, IL; see above) into the subject's mouth. The reservoir tube and the tube to the subject are connected via a 2 way Teflon valve enabling the syringe pump to draw solution from the desired reservoir into the glass syringes that are part of the syringe pump mechanism. The valve at the syringe pump will switch

direction depending on if the pump refills the syringes or pumps solution to the subject. For solution delivery, the tastant is then pressed into the Teflon tubes that are directed toward the subject. The delivering tubes are approximately 10 meters (m) in length and will be pre-filled with sample solutions so that 1.0cc of solution can be administered to the subject in 1 second. There will be 3 seconds between samples during which fMRI scanning will be performed. The syringe pump will be located in the MRI-technician room, and the tubing to the subject will run from the pump to the subject in the MRI scanner room through a port in the wall of the MRI scanning room. The syringe pump's hardware will be connected to a Laptop PC (Dell600, Dell, USA) in the technician room. This PC will control the rate of administration of the solution and the solution choice and will also be the interface between taste application/syringe pump and the MRI scanner control panel. We will use E-Prime (Psychology Software Tools, Inc., Pittsburgh, PA) software in order to coordinate the taste stimulation and fMRI scanning procedure.



Method of Taste Application

This design will be closely modeled after the design used by O'Doherty et al (O'Doherty et al., 2003). Each trial will consist of the presentation of one of five arbitrary visual stimuli (which will be abstract fractals, see Figure 3.2; we established contact with O'Doherty's group and received fractals originally used in this paradigm) followed 3 s later by either 1 ml of a pleasant sweet taste (1 M sucrose) (CS+), a neutral taste (CSneut: 25mM KCl, 2mM NaHCO3, (Francis et al., 1999, Frank et al., 2003)) or no taste (CS-). The visual stimuli will be presented on a gray background and will be removed from the screen after 3 s to coincide with taste delivery. A fixation cross will then be presented for the remainder of the trial. After a further 3 s, the next trial will be scheduled. The allocation of each stimulus to a given trial type will be counterbalanced across subjects. There will be a total of 280 trials in the experiment, 100 each of CS+ and CS- and 80 of CSneut. The whole experiment will last a total of ~28.5 min. After the first ten CS+ stimuli that will be presented and paired on each occasion with reward, in 20 out of 90 subsequent CS+ presentations, the reward will be omitted (CS+ omit). Further, in 20 presentations of the CS-, a reward will be unexpectedly delivered (CS- unexpected). The CSneut condition will be primarily included to provide a low valence rinse for the glucose taste during the experiment. The order of presentation of events will be randomized and presented to subjects using E-Prime (Psychology Software Tools, Pittsburgh, PA).

fMRI Design

Taste Reward Task: An event-related design will be used. The fMRI variables of interest are percent signal difference after: 1) taste stimulus within subject groups and across taste stimulus types, 2) within subject groups and stimulus type across expected and received vs. expected but not received stimulus, and 3) across groups within stimulus types and conditions as above.

Acquisition of images: Studies will be performed with at 3T GE MR system (GE Healthcare, Milwaukee, WI) using a standard quadrate head coil. A high-resolution, T1-weighted 3 D anatomical scan will be acquired for each subject (IR-SPGR, TR=9 ms, TE=1.9 ms, TI=500 ms flip angle=10°, matrix= 256×256, FOV=220 mm2, 124 1.7 mm thick coronal slices) for coregistration to functional data. Functional images were acquired with a gradient-echo T2 Blood Oxygenation Level Dependent (BOLD) contrast technique, with TR=14000 ms (as a clustered volume acquisition of 2000 ms, plus an additional 12000 ms silent interval), TE=30 ms, FOV=220 mm2, 642 matrix, 31 slices, 4 mm thick, no gap, angled parallel to the planum sphenoidale. Additionally, one IR-EPI (TI=505 ms) volume was acquired to improve coregistration between EPIs and the IR-SPGR. Head motion will be minimized with a VacFix headconforming vacuum cushion (Par Scientific A/S, Odense, Denmark). MR-compatible goggles (Resonance Technology, Inc, CA, USA) will be used for visual stimuli.

fMRI Image Analysis Pathway: Images will be preprocessed and analyzed using SPM12 (www.fil.ion.ucl.ac.uk/spm/). Images will be realigned to the first volume, normalized to the Montreal Neurological Institute template, smoothed at 6mm full width at half maximum Gaussian kernel. Data will be preprocessed with slice time correction and modeled with a hemodynamic response convolved function using the general linear model, including temporal and dispersion derivatives. A 128-second high-pass filter will be applied for low-frequency BOLD signal fluctuations, and motion parameters as first-level analysis regressors.

Description of how hypotheses will be tested, including neurocomputational modeling approaches. This study tests the brain response in controls compared to ILL AN women. The paradigm will test reward-learning related brain activation in response to taste stimuli. There will be a primary and a secondary analysis pathway in the paradigm.

The primary analysis pathways will use the methods described in the above fMRI analysis pathway sections. For the taste paradigm this primary analysis will identify brain region activation in response to the conditioned fractal or unconditioned sweet taste application as well as brain response when no taste is received but expected. Main effect results will be obtained for condition and subject group, as well as time activity curve

data that illustrate brain activation over time in response to the individual stimulus. *The secondary analysis pathway* includes mathematical models that try to bridge brain function and actual behavior. Computational modeling tries to explain a real world problem in a simplified model that then gets translated into a mathematical equation that can be used to correlate with brain imaging results. This serves the purpose of predicting brain response following a stimulus and depending on factors such as learning rate and received reward, different brain regions will be implicated. That is, the subject performs a specific task or Reward Paradigm and learns under what conditions reward occurs (Figure 3.5). The time course of reward experience is known by the experimenter. In the taste experiment, reward delivery or omission is part of the paradigm and recorded by the stimulus-applying computer.

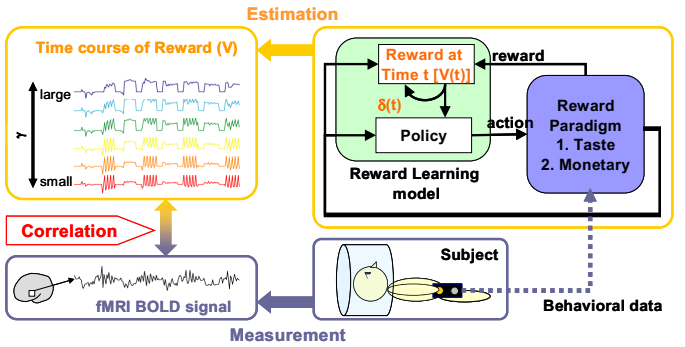


Figure 3.5. Practical application of computational modeling of fMRI data.

Use of mathematical models and testing of alternate models. Taste paradigm: For this paradigm we will use the temporal difference model as described in the past in the original description of this paradigm (O'Doherty et al., 2003). On each trial, the predicted value (V) at any time t within a trial is calculated as a linear product of the weights w_i and the presence or absence of a CS stimulus at time t, coded in the stimulus representation vector xi(t): $\hat{V}(t) = \sum_i w_i x_i(t)$.

Learning occurs by updating the predicted value of each time point t in the trial by comparing the value at time t+1 to that at time t, leading to a prediction error or δ(t): $\delta(t) = r(t) + \gamma\hat{V}(t+1) - \hat{V}(t)$

where r(t) = reward at time t. The parameter γ is a discount factor, which determines the extent to which rewards that arrive earlier are more important than rewards that arrive later on. Similar to O'Doherty's study (O'Doherty et al., 2003), we will set γ=0.99. The weights w_i will be then updated on a trial-by-trial basis according to the correlation between prediction error and the stimulus representation: $\Delta w_i - \alpha\sum_t x_i(t)\delta(t)$

where α= learning rate. We will assign six time points to each trial and use each subject's individual event history as input. On each trial, the CS (visual fractal) will be taken to be delivered at time point 1, and the UCS reward (sweet taste stimulus) will be delivered at time point 3. We will, as in O'Doherty (O'Doherty et al., 2003), use as learning rate of the model (α) two values for α: a lower learning rate (α =0.2) and a higher learning rate (α =0.7). In the original study the lower learning rate modeled best brain activation in the ventral striatum and orbitofrontal cortex. The temporal difference error δ(t) will be created for the CS and the positive and negative UCS for each time-point and an ideal activation curve will be created and convolved with the hemodynamic response curve. That curve will then be modeled to the functional magnetic resonance imaging data in order to generate brain regions that respond according to the model. Time activity curves will then be created in order to compare actual with predicted brain response over time. We cannot predict whether anorexia nervosa women will have simply reduced brain response to the stimuli or if a different learning rate will apply and explain altered learning response. This will be an empirical task during data analysis.

Reversal Learning Paradigm. We adapted a probabilistic reversal learning paradigm from Cools et al. (Cools et al., 2002). *This task has been associated with DA D2 receptor function (Jocham et al., 2009), which is why we selected it for this study design.* On each trial, subjects are presented simultaneously with two abstract visual patterns in the left and right visual fields (location randomized). On each trial, the same two patterns are

presented. One of the patterns is "correct", and the other pattern is "wrong". Using trial-and-error feedback, subjects must discover which of the two patterns is correct, using the left or right button on a button response box. On each trial, the stimuli are presented for 2000 msec within which the response has to be made. Feedback, consisting of a green smiley face and "win $1" for correct responses or a red sad face and "lose $1" for incorrect responses, will be presented immediately after the response. After feedback, the stimuli will be removed and the faces replaced by a fixation cross for a variable interval (average inter-stimulus interval is 3200 msec). Each block consists of 10 discrimination stages, and therefore, 9 reversal stages. Reversal of the stimulus-reward contingency occurs after between 10 and 15 correct responses (including probabilistic errors). The number of probabilistic errors between each reversal varies from 0 to 4. To prevent subjects from adopting a strategy such as always reversing after two consecutive errors, probabilistic negative feedback is given on two consecutive trials once during each task block. Each block lasts about 8.5 min, total number of blocks is three. Reversal Learning Task Data Modeling. The following contrasts will be modeled: (1) final reversal error trials minus correct responses, (2) other preceding reversal error trials minus correct responses, (3) probabilistic error trials minus correct responses, (4) final reversal error trials minus other preceding reversal errors, and (5) final reversal error trials minus probabilistic errors (Cools et al., 2002).

Dopamine Challenge Drugs Amisulpride and Bromocriptine

Amisulpride. Amisulpride (4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxy-benzamide has a bioavailability of 48% and a half-life of about 12 hours (Rosenzweig et al., 2002). It binds selectively (https://pubchem.ncbi.nlm.nih.gov/compound/2159) to DA D(2) and D(3) receptors in the limbic system. It reaches its peak plasma levels after 1 hour and has a second peak after 3 hours. Amisulpride does not have active metabolites (Bergemann et al., 2005). Low doses of amisulpride block presynaptic D(2)/D(3)-dopamine autoreceptors, thereby enhancing dopaminergic transmission, whereas higher doses block post-synaptic receptors, thus inhibiting DA activity (Leucht et al., 2002). In this application, we will apply a high dose to take advantage of the DA D2 receptor blocking effects of amisulpride. We are orienting our dosing on previous research using a single dose of 400mg amisulpride to block post-synaptic DA D2 receptors during fMRI (Kahnt and Tobler, 2017). That dosage showed good tolerability (Rosenzweig et al., 2002). Whether this dose is tolerable in an AN population has not been established and is a goal of this study. Establishing pharmacokinetics with plasma levels will also be important but will be part of future larger studies.

Bromocriptine. Bromocriptine is a semisynthetic, ergot alkaloid with anti-Parkinson and lactation inhibitory activities. Bromocriptine selectively binds to and activates postsynaptic DA D2-like receptors in the central nervous system (CNS), its half-life is between 2 and 8 hours, and it does not have active metabolites (https://pubchem.ncbi.nlm.nih.gov/compound/31101). Bromocriptine has a bioavailability of 28% and reaches its peak plasma level around 3 hours after ingestion (Holt et al., 2010). Bromocriptine has been used in fMRI previously and modulated reward and reversal learning (Cools et al., 2009). Effects of DA synthesis capacity will be tested in future larger studies. We will apply a single dose of 1.25mg bromocriptine, which was effective in manipulating both fMRI and behavior results (Cools et al., 2009; Lissek et al., 2018).

Special Pharmacological Considerations. Bromocriptine is an anti-Parkinson medication available in the US. An investigational new drug (IND) application to the Federal Drug Administration (FDA) has been approved, IND # 141908. Amisulpride is an antipsychotic not freely available in the US; however, it can be purchased via Sanofi France as in previous studies (Admon et al., 2017). We have received an IND from the FDA, which allows for legal import of amisulpride. Both medications have a plasma peak of 3 hours, and fMRI will begin for all sessions at that time point after taking active drug or placebo. The half-lives for amisulpride of 12 hours, and 2-8 hours for bromocriptine are short enough to allow 3 days in between studies to suffice the 5 half-lives

rule for medication elimination from the body (neither medication has active metabolites) (Bergemann et al., 2005; Holt et al., 2010; Schatzberg and Nemeroff, 2013).

Handling of Study Medication

Storage Location: UC San Diego Health Eating Disorders Center for Treatment and Research, 4510 Executive Drive, Suite 206, Rm 2-507 (PI Office, Guido K.W. Frank)

Medications will be stored in a locked cabinet in the research office of the PI. That office is embedded in the UC San Diego Health Eating Disorders Research Program office suite, which is locked. Each study drug will be labeled with the statement "Caution: New Drug—Limited by Federal (or United States) law to investigational use." (§312.6 Labeling of an investigational new drug.).

A randomization protocol will be developed ahead of the study using Research Randomizer (https://www.randomizer.org). The PI will randomize the study days for the three medications. A master file with the randomization of medication per subject will be kept by the study monitor (Dr. Bryan Tolliver).

While the tablets look slightly different, subjects will not be told what type of medication they receive on either day. Study participants have been drug naïve to any of the study medications, and we do not expect recognition. It is uncertain what specific effects each medication will have on brain function and there will therefore be no implicit bias.

Each subject will complete three MRI scan days (Study Visit 1, Study Visit 2, and Study Visit 3). On each day, the subject will consume one of the three medications (amisulpride, bromocriptine or placebo). Each subject will complete a baseline scan (P-fMRI), Amisulpride scan (A-fMRI), and Bromocriptine scan (B-fMRI) spaced 3 days apart (2 to 4 day acceptable range). This randomized study has 6 medication study arms listed below:

On the morning of each study day, the PI or other study staff not running the fMRI scanning procedures will provide the study subjects the medication as per randomization. Other study personnel that will run the fMRI scanning procedures will be kept blinded from the applied medication. This will help reduce bias by the study personnel.

There are no rater applied assessments on the study days so there is no rater bias based on the personnel end. The fMRI data are objective and thus unbiased.

The investigator will not supply the investigational drug to any person not authorized under this part to receive it. (§312.61 Control of the investigational drug.)

Neither study drug is a controlled substance. Nevertheless, the study medication packages with the investigational drugs will be in a securely locked, substantially constructed cabinet, in the PI's office, which is within an office suite that is locked and accessible only to the research personnel.

Should the subject have a serious adverse reaction that required emergency treatment, the PI would be contacted and the blinding of the medication order would be broken in order to allow adequate treatment of the patient by emergency department staff.

Each subject will be randomized to one of the following groups:

1.

Scan 1: P-fMRI
Scan 2: A-fMRI
Scan 3: B-fMRI

2.
Scan 1: P-fMRI
Scan 2: B-fMRI
Scan 3: A-fMRI

3.
Scan 1: A-fMRI
Scan 2: P-fMRI
Scan 3: B-fMRI

4.
Scan 1: A-fMRI
Scan 2: B-fMRI
Scan 3: P-fMRI

5.
Scan 1: B-fMRI
Scan 2: P-fMRI
Scan 3: A-fMRI

6.
Scan 1: B-fMRI
Scan 2: A-fMRI
Scan 3: P-fMRI

Medication dosage

Amisulpride – Single Dose 400 mg and scan 3 hours post ingestion

Bromocriptine – Single Dose 1.25 mg and scan 3 hours post ingestion

Amisulpride

Contraindications: Amisulpride's use is contraindicated in the following disease states: Pheochromocytoma, concomitant prolactin-dependent tumors (e.g. breast cancer), movement disorders (e.g. Parkinson's Disease) and lactation. Subjects will be screened during the interview with the PI to ensure they do not have any of these conditions that are contraindicated.

Potential side effects during ongoing use include fever, excessive sweating, change in heart rate, chest pain, swelling, pain, and redness in the legs, increased frequency of infections, skin allergy, seizures, restless legs, twitches in the tongue and face, trembling, excessive salivation, constipation, decreased libido, weight gain, amenorrhea, gynecomastia, agitation and anxiety, tardive dyskinesia and neuroleptic malignant syndrome.

However, we will only include drug naïve individuals and the one-time application has been well tolerated in previous studies and we do not expect problems other than maybe nausea, which will be discussed as part of the IRB consent process (Rosenzweig et al., 2002).

Amisulpride has been used in similar ways in brain imaging research.

We expect to see if at all some nausea. The other side effects are in the context of prolonged prescription and not after single dose.

Subject Stopping criteria:

If a subject were to feel so nauseous she cannot perform the study it will be stopped. If there are any other symptoms that interferes with subjects' normal functioning we will stop the study.

Bromocriptine

Contraindications: This medication is contraindicated in syncopal migraines, uncontrolled hypertension, pheochromocytoma, prolactinoma, breast cancer, hypersensitivity/allergy to bromocriptine. Participants with a history of long QT syndrome or family history of sudden death or long QT syndrome will be exclusion criteria. A history of seizures or seizure disorder is an exclusion criterion. Subjects will be screened during the interview with the PI to ensure they do not have any of these conditions that are contraindicated.

Bromocriptine is a dopamine D2 receptor agonist for the treatment of Parkinson's Disease and Type 2 Diabetes.

The medication's potential common side effects for ongoing use as per the manufacturer include nausea, headache, stomach upset, dizziness, drowsiness, feeling faint, fainting, suddenly falling asleep. Serious side effects can include heart attack, stroke, pulmonary fibrosis.

Individuals whoever took the medication before will be excluded, and the one-time application should at most be associated with nausea, headache or dizziness (Diederen et al., 2017). For each study participant only a one-time application is planned, just prior to brain imaging. The objective is to enhance dopamine transmission in the brain. We will only include drug naïve individuals and the one-time application has been well tolerated in previous studies and we do not expect problems other than maybe nausea, which will be discussed as part of the IRB consent process.

Bromocriptine has been used in similar ways in brain imaging research.

Subject Stopping criteria: If a subject were to feel so nauseous or dizzy that she cannot perform the study it will be stopped. If there are any other symptoms that interferes with subjects' normal functioning we will stop the study.

Study Duration

Altogether, assessments will take about 3 hours, the Pre-Scan blood draw and EKG will take about an hour and each brain imaging session including breakfast, medication administration and pre-scan questionnaires will take about 4 hours. Total study duration therefore is about 16 hours.

Clinical Procedures and Interventions: Not applicable, all procedures are research related, no standard of care procedures or treatment provided.

Drugs & Devices

Administration of Drugs: The drugs amisulpride (400 mg) and bromocriptine (1.25 mg) will be administered in study, 3 hours prior to the MRI scan. Both drugs are investigational drugs and the PI, Dr. Frank, has submitted and received approval from the FDA for this study (IND # 141908, see attached approval letter)

Associated Devices:

- MRI scans will be performed on a GE Discovery MR 750 3.0 T Scanner (see attached 510(k) FDA approval document (**Discovery MR750 FDA Approval - K142361.pdf**)

- EKG will be performed at the Altman Clinical Trials Research Institute.

Research Facilities and Research Procedures Performed at Facility

1. **UC San Diego Health Eating Disorders Center for Treatment and Research**: PI/Research Staff offices, subject recruitment, data storage, data analysis, consent visits, storage of medication.

2. Altman Clinical Trials Research Center (ACTRI): Pre-Scan Visit (blood draw and EKG, primary option).

3. Center for Advanced Laboratory Medicine (CALM): Blood test processing (serum pregnancy test, BUN, Creatinine, Electrolytes).
4. Sharp and Children's MRI Center: Study Visit 1, Study Visit 2, Study Visit 3

5. Medical Care San Diego: Pre-Scan Visit (blood draw and EKG, secondary option from ACTRI)

6. LabCorp: Pre Scan Visit (blood draw, secondary option from ACTRI)

Inclusion of Women and Minorities

Inclusion of Women: This project will study females with anorexia nervosa (AN) and healthy controls (HC). This project will study women only. Anorexia nervosa among males is relatively rare, males with AN have many atypical features (Andersen and Holman, 1997). Especially for a study design to be developed as in this application, it is important to keep variance, including sex, low. However, males will be included in future studies.

Inclusion of Minorities: The racial distribution of study participants with anorexia nervosa depends on individuals referred to the two treatment centers where we recruit from (UC San Diego Health Eating Disorders Center for Treatment and Research). Healthy controls will be matched to those individuals. The referral area to those treatment programs is primarily Southern California, however, the programs also draw patients nationwide. Therefore, it is reasonable to estimate subject recruitment based on the California State Census. As per the 2010 US Census data (factfinder.census.gov) the racial distribution of the population is as follows: White 57.6%, Black or African American 6.2%, American Indian/Alaskan 1.0%, Asian 13.0%, Native Hawaiian 0.4%, More than one race 4.9%; Non-Hispanic White 40.1%, Latino 37.6%. Number of subjects to be recruited is reflected in the Enrollment Table.

Minority groups present less often to treatment facilities. This may reduce access to individuals from a minority background. However, a special effort will be made for the recruitment of minority populations:
Study Population.
Eating disorders commonly occur in industrialized nations, but immigrants from nations where the disorders are rare assimilate and also may develop eating disorders, presumably because of exposure to thin-body ideals (American Psychiatric Association, 2000). There is very limited research on the prevalence of eating disorders in minority groups. However, anorexia nervosa was found to be more common in white compared to black individuals in one study (Striegel-Moore et al., 2003), and another found eating disturbances compared to Caucasian females to be equally common among Hispanic females, more frequent among Native Americans, and less frequent among Black and Asian American females (Crago et al., 1996). This highlights the need to reach out toward minority groups, in particular, Hispanic and Native American individuals. The nature of the study with many questionnaires and the specific neuroimaging tasks prohibit us to have all material and tasks in Spanish. However, it is our experience that the largest portion of individuals who might qualify for the study will be able to communicate in English. The applicant will approach those groups by providing teaching about eating disorders in those communities and provide information on the severity of illness and treatment options.
Outreach Plan.
We will make a special effort to match subjects with anorexia nervosa with healthy controls that come from similar ethnic background. There are several Universities in the San Diego area including UCSD as well as San Diego State University with multicultural student organizations.
We will carefully assess recruitment resources, incentives, and problem-solving mechanisms, and monitor the success closely.
Communication.
A main obstacle in the recruitment of minority groups is a lack of trust and knowledge (Outreach Notebook, NIMH). Thus, we will offer any of the contacted interested organizations to give a presentation regarding the study to promote understanding, awareness, and trust. The PI is already giving seminars to parents of patients with eating disorders or at local health fair events and will be more than happy to provide findings further and teaching for research staff, health care providers, participants, and their families and communities. This will increase trust and fruitful long-term collaboration.

Data Storage and Data Analysis Plan

All paper data will be stored in locked cabinets in the PI office space at UC San Diego Health Eating Disorders Center for Treatment and Research (4510 Executive Dr. Suite 330). All electronic data will be stored on the VRD server created for Dr. Frank's lab by the ACTRI (see section 16 PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT below for more details).

We will extract for both tasks mean parameter estimates from 18 predefined anatomical regions of interest (ROIs) (http://marsbar.sourceforge.net/, automated anatomical labeling Atlas, AAL (Tzourio-Mazoyer et al., 2002)) based on previous studies (DeGuzman et al., 2017): dorsal and ventral anterior insula, caudate head, gyrus rectus, medial, middle and inferior OFC, ventral striatum (O'Doherty et al., 2004) and nucleus accumbens (Breiter et al., 1997). Extracted ROI data will be analyzed using SPSS 24 (IBM statistical software) using repeated measures ANCOVA. Extracted regional brain activation parameter estimates will be tested for normality with the Shapiro-Wilk test, rank-transformed when non-normally distributed and analyzed using repeated measures ANCOVA with covariates as in previous studies to account for confounding factors such as comorbidity or medication (DeGuzman et al., 2017). Spearman's rank will be used for correlation analyses and controlled for multiple comparisons using bootstrapping (1000 samples) (Westfall, 2011). We will also conduct exploratory whole-brain analyses to test for areas of interest outside of our predefined ROIs and create PE response maps, with maps thresholded at voxel level $p<0.05$, FWE corrected (very stringent), 10 voxel minimum cluster contiguity, as well as voxel level $p<0.001$ and $p<0.05$ cluster level corrected (exploratory),

Statistical Power. This is a developmental grant application and we cannot be certain whether the study has sufficient power. However, previous studies using comparable designs and similar sample sizes showed significant within and between group results (Diederen et al., 2017). Studies from our group (Frank et al., 2018a) indicated that subject groups of 20 per group should provide sufficient power based on for instance caudate nucleus PE parameter estimate means of 67.8 (adolescent AN) and 40.2 (adolescent controls), with a mean standard deviation of 28, to reach a power of 0.8, $p<0.05$. Thus, our sample size for the fMRI study should be adequate and be able to replicate our previous results (elevated PE) and provide adequate pilot date for the new analyses in this application. A main goal of this application to investigated data distribution, standard deviation, etc. for future studies.

10. HUMAN SUBJECTS

Total Enrollment: 104 subjects (34 anorexia nervosa and 70 healthy controls)
Age Range: 18 to 29 years old
Gender: Female
Ethnic Background: All races and ethnicities
Health Status: See below

Description of Population to be Enrolled:

Subject population: Subjects will consist of female adults, ages 18 to 29 years old. We plan on completing a total of 44 subjects over 2-3 years. We expect to enroll up to an additional 60 subjects who will be disqualified during the screening process. Subjects will reflect the racial/ethnic composition of women in San Diego, CA. Total number of participants to be enrolled is 104.

Inclusion Criteria:

Healthy Controls

- Females ages 18-29 years
- Healthy body weight between 90 and 110 % average body weight since puberty.
- Regular monthly menstrual cycle
- Edinburgh Handedness Inventory Revised (EHI-R) LQ* score > +200
- English is primary language spoken

Anorexia Nervosa

- Females ages 18-29 years
- Diagnostic criteria. Current diagnosis of AN, including being underweight below 17.5 body mass index (BMI, kg/m^2), will have a severe fear of weight gain, body image distortion and absence of the menstrual cycle over three consecutive months.
- Edinburgh Handedness Inventory Revised (EHI-R) LQ* score > +200
- English is primary language spoken

Exclusion Criteria:

Healthy Controls

- Current pregnancy or breast feeding within last 3 months
- Illiterate/Blind individuals
- First degree relative with current or past eating disorder
- Current Medications other than BCP or IUD
- Contraindications to amisulpride or bromocriptine (as determined through medical history in bioscreen and PI interview) including: Syncopal migraine; Uncontrolled hypertension; Pheochromocytoma; Prolactinoma; Breast cancer; hypersensitivity/allergy to amisulpride or bromocriptine; History of long QT syndrome; Family history of sudden death or long QT syndrome; History of seizures or seizure disorder
- Past or present Axis I psychiatric disorder including substance or alcohol use disorder as determined through SCID-5 clinical interview
- Major Medical illness (as determined through medical history in bioscreen and PI interview) such as:
    - Conditions that are life threatening:
        - cancer
        - heart disease
        - stroke
        - HIV/AIDS
    - Conditions that are life threatening Conditions that cause serious disability without necessarily being life threatening:
        - stroke
        - closed head or spinal cord injuries
        - mental retardation
        - congenital malformations.
    - Conditions that cause significant pain or discomfort that can cause serious interruptions to life activities:
        - severe allergies
        - migraine
        - arthritis
        - sickle cell disease
    - Conditions that require major commitments of time and effort from care-givers for a substantial period of time:
        - mobility disorders
        - blindness
        - Alzheimer's disease and other dementias
        - chronic obstructive pulmonary disease
        - paraplegia or quadriplegia
        - Down's syndrome
        - depression
    - Conditions that may require frequent monitoring:
        - diabetes
        - conditions requiring anticoagulation treatment
        - severe asthma
        - severe allergies
        - schizophrenia and other psychotic illnesses.
    - Conditions that predict or are associated with severe consequences:
        - hypertension (associated with heart disease)
        - depression (associated with suicide)
        - diabetes (associated with blindness, kidney failure)

        - alcohol and other substance abuse (associated with intentional and unintentional injuries).
- Recent history of suspected substance abuse or a lifetime history of psychostimulant abuse and/or dependence
- Metal implants or braces (as determined through fMRI screening form)

Anorexia Nervosa

- Pregnancy or breast feeding within last 3 months
- Lifetime history of bipolar disorder or psychosis
- Illiterate/Blind individuals
- Contraindications to amisulpride or bromocriptine (as determined through medical history in bioscreen and PI interview) including: Syncopal migraine; Uncontrolled hypertension; Pheochromocytoma; Prolactinoma; Breast cancer; hypersensitivity/allergy to amisulpride or bromocriptine; History of long QT syndrome; Family history of sudden death or long QT syndrome; History of seizures or seizure disorder
- Use of an anti-psychotic or other dopamine acting medication including stimulants within the past week at time of MRI
- Recent history of substance abuse or dependence (within the last month)
- Major Medical illness (as determined through medical history in bioscreen and PI interview) such as:
    - Conditions that are life threatening:
        - cancer
        - heart disease
        - stroke
        - HIV/AIDS
    - Conditions that are life threatening Conditions that cause serious disability without necessarily being life threatening:
        - stroke
        - closed head or spinal cord injuries
        - mental retardation
        - congenital malformations.
    - Conditions that cause significant pain or discomfort that can cause serious interruptions to life activities:
        - severe allergies
        - migraine
        - arthritis
        - sickle cell disease
    - Conditions that require major commitments of time and effort from care-givers for a substantial period of time:
        - mobility disorders
        - blindness
        - Alzheimer's disease and other dementias
        - chronic obstructive pulmonary disease
        - paraplegia or quadriplegia
        - Down's syndrome
    - Conditions that may require frequent monitoring:
        - diabetes
        - conditions requiring anticoagulation treatment
        - severe asthma

        - severe allergies
        - schizophrenia and other psychotic illnesses.
    - Conditions that predict or are associated with severe consequences:
        - hypertension (associated with heart disease)
        - diabetes (associated with blindness, kidney failure)
        - alcohol and other substance abuse (associated with intentional and unintentional injuries) within the last month
- Metal implants or braces (as determined through fMRI screening form)

Rationale for excluding subjects based on gender, primary language spoken, and blind/illiterate individuals:

All subjects will be female. AN among males is relatively rare (less than 10%), males with AN have many atypical features (Andersen and Holman, 1997), and they will be subject of a different study. Our study involves several assessments and MRI tasks that have not been translated into different languages. We will only include individuals whose primary spoken language is English and will also exclude subjects who cannot read or see since the MRI tasks have visual stimuli.

11. RECRUITMENT AND PROCEDURES PREPARATORY TO RESEARCH

Selection of study population

Subjects will be recruited from UC San Diego Health Eating Disorders Center for Treatment and Research, as well through local advertisements. Subjects will reflect generally the socioeconomic and ethnic distribution of California as many patients come from the entire state.

Patient accrual

All subjects will be accrued locally. *Individuals with AN* ages 18-29 years will be recruited primarily from UC San Diego Health Eating Disorders Center for Treatment and Research, which treats per year across residential, day hospital, and intensive outpatient program more than 100 adult patients with anorexia nervosa. Willingness to participate and study eligibility ranges between 40 and 60%. We will study subjects within 1-2 weeks of admission in order to avoid the confounding effects of acute starvation or dehydration. *Healthy controls* ages 18-29 years will be recruited through local advertisements. Healthy controls will be studied during the first ten days of the menstrual cycle to keep hormonal variation low.

AN Subjects from UC San Diego Health Eating Disorders Center for Treatment and Research: Dr. Frank is an attending physician at the UC San Diego Health Eating Disorders Center for Treatment and Research program. Any eligible patients who are admitted into these programs will be screened and approached by the PI and/or research staff regarding their participation in the study. Prior to approaching the patient, Dr. Frank and/or his staff will obtain permission from the patient's individual therapist prior to making contact with the patient to describe the research opportunity. The research staff will complete the initial screening to determine eligibility. After determining initial eligibility, the PI or research staff will obtain consent from these individuals in person. All diagnostic assessments, questionnaires, and procedures will be conducted in the PI's/Research staff's private office (at the UC San Diego Health Eating Disorders Center for Treatment and Research), Altman Clinical Research Institute, or at the Sharp and Children's MRI Center (for the scanning procedures).

HC Subjects from local areas: Advertisements will be posted online and throughout the community to recruit for healthy control adults for this study. All interested individuals will contact the research staff. If interested in moving forward with the study procedures, the research staff will obtain some initial screening information over the phone to determine initial eligibility. This form will ask about current height, weight, age, gender, handedness, medication use, referral source and metal implanted in body. If the individuals meet initial criteria, the consent will be described over the phone, and a consent packet and questionnaire packet will be mailed to the individual with a stamped return envelope. The questionnaire and consent packet will consist of the following: EDI-3; SPSRQ; BDI-II; TCI; EDE-Q; STFCQ; TEPS; EEI; PBI; BCIS, SAI-ED, IUS, BIS/BAS; ACEs; fMRI screening form; Race and Ethnicity Form; Contact Information Form, ICF and HIPAA forms.

Once the coordinator receives the completed consents and questionnaires, the Initial Screening Form will be destroyed. If no packet is received within 3 months of the Initial Screening Form (**Pre-screening-Form_072219.pdf and InitialScreeningContact_072219.docx)** the Screening Contact Form will be destroyed. The research staff will score the questionnaires, as those assessments are used to further screen individuals for the study. The individual will then complete a Biological Screening Form questionnaire that goes over more details about eating and psychiatric history to rule out or confirm any eating disorder symptomatology. If at that point the subject still meets study criteria, he/she will be invited in for the in-person interview with the PI at UC San Diego Health Eating Disorders Center for Treatment and Research . At this interview, the consents will be reviewed with the subject in person to ensure they are aware of all of the study risks and procedures as well as for the PI to confirm they are eligible for the study. The subject will be given the opportunity to ask any questions about the study procedures and will be given a signed copy of the consent form. The final step before the MRIs is for the individual to complete the diagnostic assessment, SCID, which will be completed over the phone or in person (preference of the individual) by the PI or other doctoral level clinician.

If subjects pass this section and are still interested in the study, they will be scheduled for a brain scan at the Sharp and Children's MRI Center. Upon completion of the imaging study, subjects will be paid for their effort and time. Subjects will be given the opportunity to sign a consent to be re-contacted for future studies. Subjects' well-being will be monitored by the study personnel throughout the study.

Request for Waiver of Consent

Initial Screening and Identification of Potential Subjects

We are requesting a waiver of consent for the screening process.

In order to recruit eating disorder participants who are potentially eligible for the current study (e.g., adult women with AN), a waiver of consent is requested. Specifically, this will allow for an identification of adult female patients at the UC San Diego Health Eating Disorders Center for Treatment and Research program who are seeking treatment for AN, and thus meet these primary inclusion criteria for the research.

We are requesting a waiver of written documentation of consent for the Initial Screening Form (**Pre-screening-Form_072219.pdf)**. This initial screening asks verbal permission to obtain preliminary information: age, weight, use of medications, handedness, metal in body in a "yes/no" response format, and is recorded on a separate sheet from identifying information. Our procedure is to establish if prospective study participants meet minimal criteria as described before proceeding to explain the protocol and obtain written consent. If preliminary requirements are met, this information is destroyed.

Healthy Control Subjects: After the Initial Screening Form is completed, the study procedures will be explained to the subject in detail and questions about the study will be answered. If the subject would like to continue with the study, contact information including: Name, Address, Phone Number, and Email Address will be obtained (**InitialScreeningContact_072219.docx**). A consent and questionnaire packet will be mailed to the subject. Healthy Control subjects will complete the questionnaire packet and return along with the signed consent form via mail. This questionnaire packet will be used in the screening process and will help determine whether a subject is eligible for the study. Formal in-person consent will not be completed until the diagnostic in-person interview and so we are requesting an alteration of written consent documentation for the phone screenings as well as the questionnaire packets since consent will not be obtained in person prior to these screenings. The initial packet contains the following documents: Initial Study Packet Letter, Informed Consent Form (2 copies), Race & Ethnicity Form, Questionnaire Packet, Contact Information Form, fMRI Screening form.

Anorexia Nervosa Subjects: After the initial screening form is completed, the research staff will review the ICF with the subject and complete the consent process listed below in Item 11, Informed Consent.

1. The research is minimal risk.

The identification AN patients in the UC San Diego Health Eating Disorders Center for Treatment and Research Program would be of minimal risk to potential participants, as the information would be accessible only to members of the study staff directly involved with recruitment.

The Initial Screening Form is only completed after the subject gives verbal consent to ask the screening questions. The Initial Screening Form that contains eligibility information such as age and medications is destroyed after determining eligibility. The Screening Contact Form that is completed for eligible subjects will be kept in a locked cabinet and access will be restricted to Dr. Frank and his research staff.

2. The waiver will not adversely affect the rights and welfare of the subjects.

The identification AN patients in the UC San Diego Health Eating Disorders Center for Treatment would not adversely affect the rights and welfare of the subjects. As there are several ongoing studies at these programs at any given time, participants who are approached to determine their potential interest in the study would not be unduly singled out. Further, the waiver would not adversely affect the rights and welfare of the participants, as their decision whether or not to participate in the study if approached would have no effect on the care that they are receiving at the program. Importantly, the research staff will emphasize to the patients that participation in the research in no way influences eligibility to receive treatment at UCSD or elsewhere, and that participation is entirely voluntary. Participation of patients will be scheduled to reduce interference with treatment programming, and during recruitment, it will be emphasized that payment only compensates participants for their research-specific time and parking. It will also be emphasized that participants may withdraw their consent and decide not to participate at any time with no impact on their treatment at UCSD or elsewhere.

Prior to completing the Initial Screening Form, subjects give their verbal permission to complete the form. After the form is completed and eligibility is determined the consent forms are explained to the subject and the subject is asked if she has any questions and would like to continue with the study process. After the screening, only the Screening Contact Form is kept and it is stored in a secure location with minimal risk of a confidentiality breach.

3. The research could not practicably be carried out without the waiver.

The identification AN patients in the UC San Diego Health Eating Disorders Center for Treatment and Research Program could not practicably be carried out without the waiver. AN is a disorder with a relatively low prevalence in the US (0.9% of women), and as such, it would be impractical to obtain the necessary sample through community-based recruitment. The waiver of consent is requested because the recruitment of a sample of AN women could not be practically carried out at the eating disorder programs without the identification of those adult patients who are diagnosed with AN.

The Initial Screening Form is needed to determine initial eligibility criteria for the study. Our study has very strict eligibility criteria for healthy controls and therefore it is more efficient to screen interested individuals prior to having them complete all of the consents/forms/questionnaires. Since many will live outside the immediate UCSD campus area, potential research subjects would probably be unwilling to participating if they were required to come to UCSD to sign a written consent and undergo the initial screening. Also, since many healthy control subjects are excluded before completing the entire initial screening (in our previous experience about 50 percent are excluded prior to the in-person interview), 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.

Many of AN subjects will be ineligible due to handedness, age or MRI contraindications such as metal in the body and so having them complete the initial screening form before the full consent process will help minimize the time these patients have to spend away from program for research activities.

4. The subjects will be provided with additional pertinent information after participation.

There will be no additional information provided to subjects after participation in the screening procedures. Subjects who are not eligible or decline to participate in the study after the initial screening will not have contact information or PHI stored and there will be no method of contacting them regarding any pertinent information.

Request for Partial Waiver of HIPAA Authorization

Initial Screening

We are requesting a partial waiver of **HIPAA Authorization for the screening process**.

Healthy Controls: Advertisements will be posted online and throughout the community to recruit for healthy control adults for this study. All interested individuals will contact the research staff. If interested in moving forward with the study procedures, the research coordinator will obtain some initial screening information over the phone to determine initial eligibility. This form will ask about current height, weight, binge behaviors, age, gender, handedness, medication use, referral source and metal implanted in body. If the individuals meet initial criteria, the consent will be described over the phone and questions will be answered. After the subject confirms they wish to continue with the study a consent packet and questionnaire packet will be mailed to the individual with a stamped return envelope.

The questionnaire packet will consist of the following: EDI-3; SPSRQ; BDI-II; TCI; EDE-Q; STFCQ; TEPS; EEI; PBI; BCIS, SAI-ED, IUS, BIS/BAS; ACEs; fMRI Screening Form, Race and Ethnicity Form. These

forms will not contain an area for the subject to enter PHI. The consent packet will include 2 copies of the consent form and the Contact Form and will be included in this mailed forms. The mailed forms will not contain both PHI and project ID.

The individual will then complete a Biological Screening Form questionnaire over the phone that goes over more details about eating and psychiatric history to rule out or confirm any eating disorder symptomatology. If at that point the subject still meets study criteria, she will be invited in for the in-person interview with the PI at UC San Diego Health Eating Disorders Center for Treatment and Research.

At this interview, the consents will be reviewed with the subject in person to ensure they are aware of all of the study risks and procedures as well as for the PI to confirm they are eligible for the study. The subject will be given the opportunity to ask any questions about the study procedures and after a complete review of the consent forms, subjects will complete a new consent form (the consent form completed via mail as well as this newly completed consent form will be kept in the subject's folder) and the subject will be given a signed copy of the consent form.

If the individual does not meet preliminary criteria based on the answers on the Initial Screening Form, the information on the form will be destroyed. If the subject meets preliminary criteria and a consent/questionnaire packet is mailed to the subject, we will destroy the Screening Contact Form once we receive the completed submission packet back with completed consent forms and questionnaires. The Screening Contact Forms of the individuals who have not submitted consents will remain in a locked file cabinet in the locked office of the research coordinator. If no packet is received within 3 months of the Initial Screening Form, the Screening Contact Form will be destroyed. The Initial Screening Form is needed to determine initial eligibility criteria for the study. Our study has very strict eligibility criteria and therefore it is more efficient to screen interested individuals prior to having them complete all of the consents/forms/questionnaires. The PHI is necessary to collect because we send questionnaires and consent forms to these individuals. We mail the consents prior to having the individual come into the UC San Diego Health Eating Disorders Center for Treatment and Research in person because of the lengthy screening process and would like to minimize the amount of travel time required for each individual.

Anorexia Nervosa: Dr. Frank is an attending physician at UC San Diego Health Eating Disorders Center for Treatment and Research programs. Any eligible patients who are admitted into these programs will be screened and approached by the PI and/or research staff regarding their participation in the study. Prior to approaching the patient, Dr. Frank and/or his staff will obtain permission from the patient's individual therapist prior to making contact with the patient to describe the research opportunity. The Research Staff will complete the Initial Screening Form to determine eligibility. After determining initial eligibility, the PI or research staff will obtain consent from these individuals in person. In order to recruit participants who are potentially eligible for the current study (e.g., adult women with AN), a partial waiver of HIPAA authorization is requested. Specifically, this will allow for an identification of adult female patients at the UC San Diego Health Eating Disorders Center for Treatment and Research program who are seeking treatment for AN, and thus meet these primary inclusion criteria for the research.

5. Plan to a) protect identifiers from improper use and disclosure and b) destroy identifiers at the earliest opportunity.

All subjects will be assigned a "Recruitment Tracking #" prior to the Initial Screening. This number will be used in the Recruitment Log to track when a subject was approached, determined ineligible, or declined to participate (for AN subjects) and track when a research packet was sent and return and will associate the Screening Contact Form with the correct subject (for Healthy Control subjects). This Recruitment Tracking #

will be placed on the questionnaire packet that is mailed to the healthy control subjects. Only the lab manager and PI will have access to the key of recruitment tracking # and associated subject identity.

Healthy Control Subjects: After receiving the completed questionnaire and consent packet back in the mail, Dr. Frank's research staff will create a subject folder and will assign a Subject #. Only Dr. Frank and his lab manager will have the key to the Subject #s and Recruitment Tracking #s and associated subject identities.

Anorexia Nervosa Subjects: After enrolling in the study, Dr. Frank's research staff will create a subject folder and will assign a Subject #s. Only Dr. Frank and his lab manager will have the key to the Subject #s and Recruitment Tracking #s and associated subject identities.

The Initial Screening Form will be destroyed after determining eligibility. The Screening Contact Form (healthy controls only) will be kept until the questionnaire/consent packet is returned for up to 3 months. After the completed research packet and signed ICF are returned, the screening contact form will be destroyed and a subject folder will be created for the enrolled subject. If no packet is returned within 3 months, the screening contact form will be destroyed.

6. Indicate why these procedures could not a) practicably be done without the waiver, and b) be done without access to, use, or disclosure of PHI.

AN is a disorder with a relatively low prevalence in the US (0.9% of women), and it would be impractical to obtain the necessary sample through community-based recruitment. The recruitment of a sample of AN women could not be practically carried out at the UC San Diego Health Eating Disorders Center for Treatment and Research without the identification of those adult patients who are diagnosed with AN. As noted above, the privacy risk will be minimal, as the limited PHI (name, age, primary ED diagnosis) will be accessible only to members of the study staff directly involved in recruitment.

The Screening Contact Form (healthy controls) is needed in order to send the research packet.
For Healthy Controls, the PHI is necessary to collect because we send questionnaires and consent forms to these individuals. We mail the consents prior to having the individual come into the UC San Diego Health Eating Disorders Center for Treatment and Research in person because of the lengthy screening process and would like to minimize the amount of travel time required for each individual.

7. Indicate why the privacy risk to individuals whose PHI will be used or disclosed is minimal and reasonable in relation to the anticipated benefit, if any, to the individuals.

The identification of the adult patients who are diagnosed with AN privacy risk will be minimal, as the limited PHI (name, age, primary ED diagnosis) will be accessible only to members of the study staff directly involved in recruitment. Although no direct benefits are expected for participants in this research, they could potentially benefit from the psychological interviews that include a focused examination of psychiatric symptoms and history, and they may also gain satisfaction from contributing to increased knowledge on neurobehavioral mechanisms of AN. Participants who are enrolled into the study will be asked to sign a HIPAA authorization form along with the informed consent document, while the PHI for individuals who either choose not to participate or who are found to be ineligible will be immediately and permanently deleted. Access to

information including age, sex, and ED diagnosis is requested for this waiver, as these details will allow for the targeted recruitment approach that is necessary to practically and feasibly carry out this research.

The Screening Contact Form (healthy controls only) is kept in a locked cabinet that only Dr. Frank and his research staff have access to.

The identification key to the Recruitment Tracking # and Subject # will be stored in a password-protected databases on secured computers that are accessible only to the PI and lab manager.

8. Indicate what PHI will be used and who will access, use or disclose the PHI.

The identification of the adult patients who are diagnosed with AN is limited to the following PHI: Name, Age Primary ED diagnosis and history. This PHI will only be used to identify which patients might be eligible. Only research staff directly involved in this study will have access to this information.

The Screening Contact Form (healthy controls) will contain: Name, Address, Email, and Phone Number. This information will be used to mail the consent/questionnaire packet to the potential subject. Mailed packets will not contain materials that have both PHI and project ID.

The Recruitment Tracking # and Subject # Identification Keys will contain the name and associated Tracking and Subject #s. Only the PI and Lab Manager will have access to this PHI.

12. INFORMED CONSENT

Consent Procedures

AN subjects from UC San Diego Health Eating Disorders Center for Treatment and Research

As a provider of the UC San Diego Health Eating Disorders Center for Treatment and Research, Dr. Frank has a clinical relationship with the patients in treatment at these programs. He and his staff will screen new admissions to determine potential subjects. Prior to approaching the patient, Dr. Frank and/or his staff will obtain permission from the patient's individual therapist prior to making contact with the patient to describe the research opportunity.

Dr. Frank's research staff will approach potential subjects and get their verbal consent to complete the initial screening (Pre-ScreeningForm_072219.pdf). Prior to approaching any potential subject, the research staff will contact the patient's primary therapist After completing the initial screening form, the research staff will destroy the pre-screening form. If the subject is eligible, the research staff will review the consent forms and complete the consent visit as outlined below:

1. The person obtaining consent (i.e. the research assistant or PI) will be trained to answer questions and explain the study and document the consent process. Any person involved in the consent procedures will have completed the UCSD HRPP educational requirements.

2. The setting in which the consent will be obtained will be in a clinic or research setting (UC San Diego Health Eating Disorders Center for Treatment and Research, in a quiet room with no distractions. The subject will have unlimited time to read and ask questions about the study and the consent process. Patients in this research study will be informed about the purpose and the duration of the current study. Informed consent will be obtained once the subject has been informed and wants to participate in the investigation using the attached consent form. The PI or research assistant will go through the consent form with the subject and will obtain written consent.

3. The subject's comprehension and autonomy will be assessed by asking the subjects to explain in their own words the study procedures in order to assure the understanding and ability to consent.

Consent and willingness to participate will be confirmed by staff in person prior to conducting any other study procedures. The PI who has extensive experience with screening subjects for similar studies will perform initial screening and assessments. However, HRPP certified staff will also be trained to perform screening and subject enrollment. Subjects will be explained the study, will have time to review any consent papers and will sign when in the office and after further discussion of the study participation.

Subject's comprehensive autonomy is assessed by having subjects report back on how they understand the study in their own words and the PI /study personnel will judge if subjects understood correctly. After all questions about the study are answered the consent form will be signed by the subject and a copy of the consent will be provided to subjects. The consent will be kept in the participating subjects' files.

In addition to the general consent, participants will also be given the option on the consent forms to opt-in to allow their data to be shared with other research teams at the UC San Diego Health Eating Disorders Center for Treatment and Research who are studying eating disorders.

A copy of the consent will be provided for the subject.

The consent process will be documented in the subject's summary checklist.

Healthy Controls from the Community

Potential subjects will voluntarily contact us about participating in this research either by phone or in writing. The study will be advertised on posted flyers and research web pages that will include our research office contact information.

Since many will live outside the immediate UCSD campus area, potential research subjects would probably be unwilling to participating if they were required to come to UCSD to sign a written consent and undergo the initial screening. Also, since many subjects are excluded before completing the entire initial screening (in our previous experience about 50 percent are excluded prior to the in-person interview), 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.

We are requesting a waiver to document consent for the initial screening process only. A brief, verbal screening tool will be used (**Pre-screening-Form_072219.PDF and InitialScreeningContact_072219.docx**)

This Initial Screening Form asks the individual for verbal permission to obtain preliminary information: age, height, weight, gender, binge eating behavior, handedness, implanted metal, use of medications, and referral source as well as contact information of the individual. Our procedure is to establish if prospective study participants meet minimal criteria as described before proceeding to explain the protocol and obtain written consent.

After the research assistant determines the subject meets the initial criteria, the study procedures will be described over the phone to the subject, questions will be answered, and if the subject agrees a questionnaire packet and consent form will be mailed to the subject.

We will add to the information sheet, which has name and address, to document that verbal permission was obtained to obtain name/address. The questions we are asking during this initial screening are 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, email and phone number if the subject is willing to do so (**InitialScreeningContact_072219.docx**).

We are requesting a waiver of written consent and waiver of HIPAA authorization for the pre-screening process. As stated above, the research staff will complete a phone screening to determine initial eligibility criteria **(Pre-screening-Form_072219.pdf).** Once the subject is determined eligible or ineligible this screening form will be destroyed. After obtaining verbal consent from the subject to continue with the study, the research staff will obtain contact information (**InitialScreeningContact_072219.docx)** and will send an initial packet to them via mail that contains the following: Initial Study Packet Letter, Informed Consent Form (2 copies), Race & Ethnicity Form, Questionnaire Packet, Contact Information Form, fMRI Screening form.

If a subject passes the screening processes (Initial Screening Form, Questionnaire Packet in acceptable range, Phone Bioscreen), the subject will be invited in for the diagnostic assessment and in person interview with the PI. At this visit, the consent form will be reviewed with the subject, questions will be answered, and comprehension of study procedures will be assessed. If the subject agrees to continue, she will complete a new consent form that will be stored in the subject folder along with the consent form completed via mail that accompanied the questionnaire packet. The PI who has extensive experience with screening subjects for similar studies will perform initial screening and assessments. However, HRPP certified staff will also be trained to perform screening and subject enrollment. Subjects will be explained the study, will have time to review any consent papers and will sign when in the office and after further discussion of the study participation.

Subject's comprehensive autonomy is assessed by having subjects report back on how they understand the study in their own words and the PI /study personnel will judge if subjects understood correctly. After all questions about the study are answered the consent form will be signed by the subject and a copy of the consent will be provided to subjects. The consent will be kept in the participating subjects' files.

The request for waiver of documented consent for the initial screening portion meets the following criteria from 45 CFR 46.117(c):
ii. That the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

For the AN subjects, the initial verbal screening to determine eligibility will be destroyed right after completing the screening.

For the HC subjects, the initial verbal screening (**Pre-screening-Form_072219.pdf**) to determine eligibility will be destroyed right after completing the screening. If eligible and subject agrees, they will provide contact

information including name, address, phone, email (**InitialScreeningContact_072219.docx)** where we will send the research packet to them. This initial screening contact information form will be kept in a locked cabinet accessible by only Dr. Frank and his research staff.

Data Sharing and Concurrent Enrollment in Protocols

Some enrolled subjects may be enrolled in another NIMH funded protocol conducted by Dr. Frank's lab. If the subject signs up for multiple, there will overlap in questionnaires and diagnostic interviews. In order to minimize the amount of time the subject has to spend doing the study procedures and also to reduce redundancy in study procedures, subject's data will be shared between studies if they are enrolled in both protocols.

Authorization procedures

An authorization will be obtained at the time of consent procedures by the PI or trained study personnel.

A signed and dated copy of the authorization form (included in the combined consent/HIPAA authorization form) will be provided to the subject.

The data acquired will be stored in a locked file cabinet in a locked UCSD research office. A completed authorization form with study specific information for HRPP review will be retained in the subjects' files.

The authorization portion of the consent forms include UC San Diego Health Eating Disorders Center for Treatment and Research as an entity to receive protected health information for only patients enrolled in their program. This information includes scheduling of MRI scans and study procedures so that it does not conflict with program time as well as any adverse reactions or events that occur during the testing procedures that may impact their treatment in program.

After signing a written consent/assent, all subjects will complete written self-assessments, as well as structured interviews with the research assistant or PI with information stored on a computer. Data from the in-person interview will be recorded in writing and stored with the subject's research chart. The Edinburgh Handedness Inventory will assess the dominant hand for analysis of handedness and recorded in the subject's research chart.

After the subject has completed all study procedures and their behavioral data has been entered into our electronic database, Dr. Frank's research staff will create a Certified Electronic Copy of the subject's paper documents which include the signed ICF, questionnaires, contact information, scan assessments, and diagnostic interview and the paper documents will be shredded. This Certified Electronic Copy will be kept on a secure UCSD server that is password protected and encrypted. Only Dr. Frank and his research staff will have access to these documents.

Study Assessments: All subjects will be administered the structured clinical interview for DSM-5 diagnoses (SCID) by a PhD level Psychologist or the PI. All participants will complete the Eating Disorder Inventory-3 (Garner 2004, Cumella 2006), revised Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ) (Torrubia, Avila et al. 2001), Beck Depression Inventory (BDI-II) (Beck, Steer et al. 1996), Temperament and Character Inventory (Cloninger, Przybeck et al. 1994), Eating Disorders Examination Questionnaire (EDE-Q) (Fairburn 2005), State and Trait Food Cravings Questionnaire (STFCQ, craving self-report) (Cepeda-Benito, Gleaves et al. 2000, Moreno, Rodriguez et al. 2008, Van den Eynde, Claudino et al. 2010, Van den Eynde, Koskina et al. 2012), Temporal Experience of Pleasure Scale (TEPS, food and non-food

anticipatory and consummatory experiences of pleasure) (Gard, Germans-Gard et al. 2006), Eating Expectancy Inventory (EEI, cognitive expectations for eating) (Hohlstein, Smith et al. 1998), Parental Bonding Instrument (PBI, parental bonding may be a moderator of brain dopamine release) (Parker 1989, Pruessner, Champagne et al. 2004), Schedule for the Assessment of Insight for eating disorders (SAI-ED) (Konstantakopoulos et al., 2011), Adverse Childhood Experiences Questionnaire (ACEs, Feelitti et al, 1998), Behavioral Inhibition Scale/Behavioral Activation Scale (BIS/BAS, Carver & White, 1994), Intolerance of Uncertainty Scale (IUS, Freeston et al, 1994).

13. ALTERNATIVES TO STUDY PARTICIPATION

There is no direct benefit to the participant. The alternative to study participation is to not participate in the study.

14. POTENTIAL RISKS

Potential Risks

It is our experience that individuals with eating disorders including AN are willing to cooperate with such studies. Over the past 12 years we have recruited and studied close to 400 subjects and had no incident that would indicate any risk. The studies were not psychologically traumatic to adolescents or adults in the past. We do not feel that these studies pose any significant psychological risk for patients or control study subjects. Many measures are in place to assess and avoid risks.

Computer Testing/Interviewing. The main risks associated with these procedures are fatigue and irritability with the testing procedure. The investigator and research associates are trained to frequently ask the subjects about their willingness and ability to continue with testing. If a subject expresses concerns about continuing with testing, the investigator has instructed the research associate to stop testing, offer a break, or, if the subject is not willing to continue, to terminate the testing session. Overall, however, previous studies have not resulted in any significant discomfort or anxiety expressed by the participating subjects.

Social Risk. There is no social risk associated with the participation in this study, all results will be kept strictly confidential and will not be used to influence any clinical decision making. There are no legal risks associated with these paradigms. There are no other known risks associated with participation in this study. AN patients will continue to receive their current treatment, which is not being suspended or modified in any way for research purposes.

fMRI Brain Imaging. There are two major sources of risk. First, there could be risk from the magnetic field in the MR scanner, second the subject may experience discomfort being in the confined environment of the scanner (claustrophobia) with exposure to noise, and third, the subject may experience discomfort carrying out the task. The Sharp & Children's MRI Center is run by full time trained technicians who have extensive experience and training to conduct safely the here proposed studies. In addition all research personnel involved in this study is/will be trained in MR safety by the imaging center staff.
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. Conventional MRI uses 1.5 Tesla (T) magnets. At our 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. The scanning sequences applied are within the FDA guidelines for human MR scanning.

In addition, every subject undergoes two extensive safety screenings to determine whether he/she has any implanted materials or braces and dental retainers that may pose a risk. This screening will be done once the subject is consented, and another time on each imaging study day. If there is any doubt about the nature of any implanted material, or any other indication of contraindication of MR scanning, the subject will not be scanned. Every female subject will have a urine pregnancy test that is reviewed prior to scanning and if there is indication of pregnancy the subject will be excluded.
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 have relatively low power depositions. In the future, pulse sequences with higher RF power depositions may be developed, but we will ensure 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 rates of change sufficient to produce severe discomfort or painful stimulation" is used. All of the fMRI studies performed in the past on our 3T system are well below this threshold.

Confined space and acoustic noise. The small space in the MRI scanner can cause discomfort and feelings of being trapped. This has occurred occasionally in our studies (about 2-3% of studies). Acoustic noise is 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 of our studies, subjects will wear ear plugs to reduce the noise to a comfortable, safe level. Additionally, head phones will be placed over the ears. The FDA guideline for a significant risk due to acoustic noise is "peak acoustic noise over 140 dB", and we will ensure that the acoustic levels remain well below this value.

Discomfort during the task. It is possible that subjects experience discomfort from the manifold placement or taste stimulus application. We have not received such complaints in our previous studies but we are very aware of such a possibility and confirm with subjects when we communicate with them during the experiment that the manifold placement is pleasant, not irritating and that subjects are comfortable.

Potential Risk of Loss of Confidentiality. Since this study includes medical and psychological assessments as well as height, weight and urine pregnancy test assessment, there is the potential that this information may not be kept confidential (for instance by theft of study material). The investigator team will make every effort to keep all information confidential. All study material will be stored in locked cabinets in the UCSD sponsored facilities. Furthermore, a unique study number will be used for each person in data sets and spreadsheets that do not readily identify a name. The identifying name information containing material will be locked. The information shared over the EMA server will not include identifying information. This system is secured as described above and only the PI and his staff will know the identity of each subject registered in the system.

Medical or Psychiatric Emergencies. It is possible that during the assessment procedures or any other time during the study any study personnel becomes aware of medically or psychiatrically concerning knowledge, including suicidality or homicidality, or child abuse or neglect. Our consent forms include that action will be taken and that we may have to contact the authorities. We have a specific procedure in place that will ensure subjects' safety and well-being.

Challenge Drug Application: Two dopamine challenge drugs will be applied, amisulpride and bromocriptine. Each one of them will be applied only once. Bromocriptine is a dopamine D2 receptor agonist for the treatment of Parkinson's Disease and Type 2 Diabetes. The medication's potential common side effects for ongoing use as per the manufacturer include nausea, headache, stomach upset, dizziness, drowsiness, feeling faint, fainting, suddenly falling asleep. Serious side effects can include heart attack, stroke, pulmonary fibrosis. Individuals whoever took the medication before will be excluded, and the one-time application should at most be associated with nausea, headache or dizziness (Diederen et al., 2017). Amisulpride is a selective dopamine D2 receptor antagonist used frequently in Europe and other countries for psychotic disorders. Potential side effects during ongoing use include fever, excessive sweating, change in heart rate, chest pain, swelling, pain, and redness in the legs, increased frequency of infections, skin allergy, seizures, restless legs, twitches in the tongue and face, trembling, excessive salivation, constipation, decreased libido, weight gain, amenorrhea, gynecomastia, agitation, anxiety, tardive dyskinesia and neuroleptic malignant syndrome. Similarly to bromocriptine, we will only include drug naïve individuals and the one-time application has been well tolerated in previous studies and we do not expect problems other than maybe nausea (Rosenzweig et al., 2002).

Blood Draw. The main risk with the blood draw is some bruising when the needle goes into the vein and bruising at the needle site a day or two after the blood draw.

Electrocardiogram. The main risk is skin rash where electrodes are placed.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

Adequacy of Protection against Risks

Recruitment and Informed Consent. The Research Assistant will do a brief UCSD Institutional Review Board approved screening for age, height and weight over the phone for controls and for AN subjects in their respective treatment program together with the PI. All study participants are repeatedly informed that they can leave the study at any time and that participation or study decline will have no effect on treatment.

Consent Procedures: Consenting procedures will be HIPPA compliant. The objectives, procedures, and a clear statement explaining risks and benefits of the study (as described in the consent form) will be presented to each potential subject prior to her/his entry into the protocol. After the study has been explained to the study subjects, questions will be asked and elicited in order to ascertain that the subjects comprehend the procedure to which they are being asked to give consent to.

Computers. The computers and the interfacing devices will be checked regularly for proper and safe operation. Our computers are also embedded in the UCSD computing network and this ensures strict safety rules and data protection by fire walls.

Assessments and Tasks. AN and HC subjects will be carefully screened and subsequently informed about the fact that the principal investigator will be available at all times during the experiment. Subjects are informed that they may end test sessions at any time and that participation in this research is voluntary. Examiners will be clinically trained to be sensitive to signs of stress, anxiety, or fatigue so that testing will be immediately terminated should any subject experience signs of discomfort.

Social Risk and Loss of Confidentiality. All staff that participates in this study is HIPAA and conduct of research trained according to the UCSD guidelines. A number system will be used for identification of subjects,

and the key that links the number to the subject will be kept in a password-protected Excel file, which will be stored on an encrypted laptop that is locked in a cabinet, so to not expose the file to potential attacks to networked computers.

Brain Imaging. 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 study.
During scanning the subject holds an emergency button in her hand that he/she can push and get help at any time during the experiment. If at any point during the scanning the subject expresses increasing discomfort, the PI, Research Assistant or Sharp and Children's MRI Center staff 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 Brain Imaging Center staff following University rules and procedures.
Heating from radiofrequency (RF) pulses: The fMRI 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 with respect to potential heating effects. The physicists at the Sharp and Children's MRI Center regularly test pulse sequences on phantom objects for heating effects.
Acoustic noise: Subjects will wear earplugs and 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 occurs, the experiment will be terminated immediately.

Blood Draw. Trained nursing staff at ACTRI (or Medical Care San Diego and LabCorp) will perform all blood draws (certified phlebotomists).

Medical or Psychiatric Emergencies. If during the assessment procedures or any other time during the study any study personnel becomes aware of any medically concerning information or psychiatric emergencies.

Challenge Drug Application: After application of study drugs we will assess participants' well-being. Participants will be informed during the consent process about possible side effects and subjects can withdraw from the study at any time. Both medications have been used in single drug applications before and were well tolerated. Based on our exclusion criteria, no individual who took the medication in the past should be allowed to be in the study due to rule out of major medical illness including Parkinson's or diabetes. However, we will screen for lifetime use of either of those drugs to exclude the risk of allergic reaction due to prior exposure.

The feasibility procedures will also include assessment of side effects from the medication. In case a study participant was to have a serious reaction (such as anaphylaxis) 911 would be called immediately and the person transported to the medical emergency room that is located about 500 yards away on campus. The study personnel are trained in basic life support.

Cardiac conduction abnormalities have been noted in individuals with anorexia nervosa. To ensure patient safety, we will only include patients in our study that are considered medically stable by the Medical Director at UC San Diego Health Eating Disorders Center for Treatment and Research (i.e. normal EKG and lab electrolyte values). If the staff at those facilities does not feel their patient is stable enough to complete the fMRI scan, the study will be postponed until she is stable, or the patient will be withdrawn from the study if timing does not allow the scan to be postponed. Study participants will have their blood pressure and heart rate taken prior to study drug administration as well as at the end of each study day. In case there is a significant drop in blood pressure indicating orthostasis during the study or due to the study medication, we will keep study participants in our research space and provide them with fluids and will recheck the blood pressure until

it has normalized. In case there were ongoing indication of orthostasis we will refer the participant to the local emergency room on campus.

Electrocardiogram. The risk is minimal. Any skin irritation from the electrode placement usually lessens within a few minutes of removing the electrode.

Data and Safety Monitoring Plan

Data will be analyzed as above for brain imaging data. The behavioral data will be analyzed using SPSS statistical software on password protected computers. The PI will perform data analysis with some research assistant support.
Subjects will be continuously monitored during their study participation by asking about how subjects feel and if they have any problems while in the study. However, in case of unexpected effects, the PI will report those to UCSD HRPP.

Suicidal Ideation: In the proposed study populations there is the possibility of mood disturbance including suicide and extreme emotional distress or anxiety. The questionnaires applied will give indication about mood states as well as will the diagnostic procedures conducted with research staff and the PI. Those questionnaires and diagnostic assessments will be reviewed by the PI and any other involved study personnel will be trained to contact the PI immediately in case there were behavioral responses that indicate danger.
For Healthy Control Subjects, should the PI discover suicidal ideation from their answers on the questionnaires, he will contact the subject directly and assess suicidality over the phone or if needed will request a health check up through the police department. If acute suicidality is discovered during the in person interview, then the PI will contact emergency medical services to escort the patient to the emergency room. For AN Subjects, the PI will inform the patient's individual therapist and treatment team of suicidal ideation. Because the AN patients will all be in a highly supervised treatment environment, they are assessed regularly throughout the day for suicidal ideation. Dr. Frank will work with the patient's therapist to determine the appropriate course of action which might include emergency room care if acutely suicidal.

During consent procedures, participants will be informed of staff's responsibility to report any disclosure of current child abuse or neglect or intent to hurt oneself or someone else to the appropriate officials. Appropriate services will be informed if such information regarding abuse or neglect is made available. Research staff will review each subject with the PI and is educated about decisions related to reporting child neglect and abuse and have been and will be trained to consult the hospital and/or university legal counsel if necessary for guidance.

There will be no independent data safety monitoring board. Subject files will be stored in a research-designated locked file cabinet, in a locked office at UC San Diego Health Eating Disorders Center for Treatment and Research. All scored assessments and study results will be stored on password-protected computers and stripped of readily identifiable information.

As a mandatory reporter, the PI will contact social services in case we learn about any current case of child abuse. The study population is in the adult range so this will be a less likely scenario, however, we will report in case we learn about child abuse.

Plans for monitoring study conduct: the protocol will be conducted according to a time line. Prior to study initiation a detailed procedure protocol will be established and for every subject enrolled there will be the subject flow chart that is followed. This will ensure that all study parts will be adhered to. Each time a study

part has been completed, the PI or study personnel will check off and initial. This flow chart will also serve as a scheduling tool with notes for upcoming study parts such as the fMRI scan.

Things That Must Be Reported to the Authorities

During consent procedures, participants will be informed of staff's responsibility to report any disclosure of current child abuse or neglect. The scale that examines these issues is a retrospective assessment of abuse history current adults may or may not have been exposed to as children. Appropriate services will be informed if such information regarding *current* abuse or neglect is made available. Research staff has been, and will be on a continuous basis during case review with the PI, educated about decisions related to reporting child neglect and abuse and have been and will be trained to consult the hospital and/or university legal counsel if necessary for guidance.

16. PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT

Privacy and Confidentiality

Setting. All consenting procedures will be conducted in the private offices of the PI and research staff at the UC San Diego Health Eating Disorders Center for Treatment and Research. The Pre-Scan Study Visit will take place at ACTRI (primary option) or at San Diego Medical Care or LabCorp facilities (secondary option). Study Visit 1, Study Visit 2, and Study Visit 3 procedures will be conducted at the Sharp and Children's MRI Center.

Safeguards to Protect Subject Confidentiality. Sensitive information about a subject's medical and psychiatric history including substance abuse and treatment histories are collected as part of this research study. To protect the privacy we will keep all subject folders in locked file cabinets in the locked offices of the PI and research coordinator. Only the PI and research staff will have access to these files. In addition, the possible risk of loss of confidentiality is explained to the subject as a possible risk and he/she has the option of not participating in the study. Information about current psychiatric illness and current medication use is collected as research data. In addition, information about drug abuse and dependence is collected as part of the study. The patient populations included in this research study include participants who have been diagnosed with a medical or psychiatric condition. While this information is considered sensitive, it is necessary to collect this data to provide an accurate description of our subject populations and use as a comparison to our healthy control populations. All subjects are told in the consenting process that they can choose to not answer any question. While they do have the right to refuse to have this sensitive data collected, some of the sensitive data are necessary to collect for the outcome of the study (including substance use, medical conditions, etc.), therefore, the principal investigator may choose to withdraw or not include any participant who chooses to not have this sensitive data collected.

Data Storage and Access

When a participant enrolls in the study, a subject folder will be created that contains original documents of the ICF and HIPAA forms as well as all of the self-assessment forms and scan day forms. Payment information will also be kept in the subject folder. The folders will be kept in a locked cabinet of Dr. Frank or his research staff and access will be limited to his research staff working on this study. In addition, a subject number will be assigned to each participant and the key that contains the subject's identity will be kept electronically in a password protected file by Dr. Frank's lab manager.

After the subject completes the study procedures (after Scan Day 2), the research staff will make a certified electronic copy of all of the subject's paper data which include: Signed ICF, Consent documentation, Signed HIPAA Authorization, fMRI Screening Form, Race & Ethnicity Form, Contact Information Form, Questionnaire Packet, Scan Day Assessments, Subject Face Sheet, Subject Medication Effects Log, Lab and EKG Results, and Payment information. This certified electronic copy will be stored on an encrypted and regularly backed up server at the UC San Diego Health Eating Disorders Center for Treatment and Research and access to these files will be limited to Dr. Frank and his research staff. Other electronic files to be stored on this encrypted server (de-identified): SCID 5 assessment and brain images.

The behavioral and questionnaire data will be entered into a password protected excel file. This file will contain no PHI.
The brain imaging procedures will be performed at Sharp & Children's MRI Center. This center will perform the scans as a fee for service facility and a Letter of Agreement will be established between the Department of Psychiatry at UCSD and the Sharp & Children's MRI Center. After the completion of the MRI scans, de-identified brain images will be transferred through CD or direct transfer to a landing site that will be created by the ACTRI and from there transferred to the VRD environment created for our lab by ACTRI. The data transferred will not contain PHI and access to the images will be restricted to Dr. Frank and his research staff.

Once complete, the paper documents will be securely destroyed and only the certified electronic copy and de-identified data will be kept.

All data collected under this Research Plan will be stored on the ACTRI VRD Environment created for Dr. Frank and his lab.

17. POTENTIAL BENEFITS

There is no direct benefit for individuals participating in this study. However, individuals with AN participating in this study may benefit from the screening procedures that include a careful examination of the subject's psychiatric condition. They may request a copy of the in-depth diagnostic assessment that they may share with their provider if they wish or review with the PI.

18. RISK/BENEFIT RATIO

Risk/Benefit Ratio: This study involves questionnaires, in person assessments, a blood draw, EKG and 3 fMRI scans with administering a single dose of 2 medications and placebo to subjects and there are potential risks to taking the medication as outlines above. Anorexia nervosa (AN) is the most deadly disease among the psychiatric disorders (Sullivan, 1995) and no medication has been approved to this date to treat the disorder. There is a potential benefit of investigating the concept that direct receptor acting medication can ameliorate excessive brain dopamine related reward response in AN and thus could in fact become an important treatment for this disorder; therefore, we feel the potential benefits outweigh the risks involved in this pilot study.

Importance of the knowledge to be gained

The results from this project will
(1) provide information on how feasible brain imaging and dopamine challenge drug application is in anorexia nervosa.

(2) provide important new information on how dopamine D2 receptors are involved in prediction error response and reversal learning within anorexia nervosa and healthy control groups.
(3) provide pilot data whether healthy controls or individuals with anorexia nervosa can be distinguished by dopamine receptor-driven brain response.

Anorexia Nervosa has the highest mortality rate among the psychiatric disorders and is associated with an immense amount of suffering for the patients and their families. The results of this study will help us identify how dopamine receptor function is involved in altered brain activation and behavior response in anorexia nervosa. What this may help is identify targets for pharmacological intervention. We believe that the benefits of the study by far outweigh the potential risks.

19. EXPENSE TO PARTICIPANT

None

20. COMPENSATION FOR PARTICIPATION

Questionnaire Packet & Initial Screenings: $25
Diagnostic Assessment: $25
Pre-Scan Study Visit: $25
Study Visit 1, 2, 3: $150/Study Visit
Reimbursement for Parking at ACTRI Pre-Scan Visit: $5

Total Compensation for Study: $530

Although rare, it is possible that the MR scanner may have errors or problems or there are personnel issues, or problems with the taste pump, etc. that prevent us from completing the scheduled study visit day. Sometimes these issues are not known until the start of the scan after the subject has already completed many of the questionnaires, taste test, etc. If the timing of the scan allows us to reschedule one/both of the Study Visits, we will ask the subject if they are willing to come back to complete an additional study visit day and will compensate them $100 for the incomplete study visit in additional to the amount they receive for the regular study procedures.

Ineligible subjects or subjects who withdraw from the study prior to Study Visit 1 will receive an electronic gift card in the amount of $25, $50 or $75 (depending on what stage of the study the subject completed) to Amazon or Target.

In the consent form the subject is informed that compensation in this study is considered taxable income and after the subject passes the Screening Visit, the subject will be given instructions on how to register herself on Payment Compass. The subject will be reminded that research staff will submit a request for payment to UCSD after they complete the study and that if they are excluded or withdraw prior to the first MRI scan (Study Visit 1), they will be compensated in the form of an electronic gift card.

If a subject did not choose to register herself on Payment Compass (or those individuals who do not have easy access to complete the Self-Registration process on Payment Compass, such as AN subject in a treatment program with limited internet access), we will have the subject complete a paper W9 form with their social security number and contact information. The research staff will then register the subject in Payment Compass and submit the payment request to UCSD after the completion of the study.

The payment is to serve as an incentive for subject participation and to reimburse travel, study time and expenses. Although the individual subject may not receive any benefit beyond financial remuneration, the data

generated by this project could significantly enhance our knowledge about altered brain function with respect to anorexia nervosa and pave the way for new clinical measures and treatments for those disorders.

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

Principal Investigator: Dr. Frank, MD will serve as the Principal Investigator on this study. As the individual who is primarily responsible for the study he will oversee all aspects of research including regulatory, grant submissions, recruitment, consenting, study procedures, data entry and data analysis. Dr. Frank is employed by UCSD and is funded through the R21 grant of this study.

Lab Manager: Ms. Megan Shott, BS, will serve as the Lab Manager for Dr. Frank. She will oversee the day to day tasks of the lab and this protocol including recruitment, consenting, study procedures training and administration, data entry, and data analysis. She will also be responsible along with Dr. Frank to ensure the study meets all regulatory requirements and ensure that all staff members have adequate training to run the study. Dr. Frank may assign Ms. Shott other duties via the Delegation of Duties Log. Ms. Shott is employed by UCSD and is funded through the R21 grant of this study.

Staff Research Associates and students (TBD). Dr. Frank may employ one or more staff research associates or students who will be responsible for recruitment, running study procedures and data entry as well as other duties assigned by Dr. Frank via the Delegations of Duties Log. These individuals will be employed by UCSD and funded through the R21 grant of this study.

Skylar Swindle, BS, will act as a staff research associate and will be responsible for recruitment, running study procedures and data entry along with other duties assigned by Dr. Frank via the Delegations of Duties Log. She is employed by UCSD and funded through the R21 grant of this study.

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23. FUNDING SUPPORT FOR THIS STUDY

This study is funded by the National Institute of Mental Health R21MH120475. Project Period: 8/1/2019 – 4/30/2021.

UCSD Fiscal Staff Info:

Fnu Clarissa
Fund Manager
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24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

Not applicable.

25. INVESTIGATIONAL DRUG FACT SHEET AND IND/IDE HOLDER

Drug Names: Bromocriptine mesylate; Amisulpride
IND #: 191908
IND holder: Guido K.W. Frank

Dr. Frank and the Lab Manager will be responsible for monitoring the study in the following ways:

Medication Handling and Storage

- Per 21CFR §312.6 Labeling of an investigational new drug, medications will be stored in a locked cabinet in the research office of the PI. That office is embedded in the UCSD Eating Disorder Research Program office suite, which is locked. Each study drug will be labeled with the statement "Caution: New Drug—Limited by Federal (or United States) law to investigational use."
- Per 21CFR §312.61 Control of the investigational drug, the investigator will not supply the investigational drug to any person not authorized under this part to receive it.

Subject Level

- Dr. Frank will review all Pre-Scan Blood Test and EKG results to ensure subject's medication risks are minimized.
- Research staff will complete symptom checklist on each Study Visit day and will document the following: Documentation of blood pressure & heart rate pre-medication administration and post scan; symptom checklist including: nausea, headache, dizziness, tiredness, other. Research Staff will report these values to Dr. Frank and the Lab Manager at the end of the Study Visit.
- Research staff will contact Dr. Frank and/or Lab Manager via phone if there is a significant drop in blood pressure (20 or more points systolic) after the medication administration or significant symptoms that the subject feels keep her from continuing the study.
- Dr. Frank or Lab Manager will confirm with research team that all unexpected or adverse events (AEs/SAEs) have been reported to the IRB and FDA in the required timeframe.
- Dr. Frank or Lab Manager will review each subject folder after the subject completes the study to ensure required documentation and procedures have been completed.

Study Level

- The Lab Manager will prepare monthly aggregate reports of the adverse events, serious adverse events, and unexpected events and will review with Dr. Frank.
- Dr. Frank will review monthly aggregate reports to determine safety of the study medications and whether the study is safe to be continued.

Per FDA Approval, Dr. Frank will be responsible for the following reporting requirements:

- Dr. Frank will report any unexpected fatal or life-threatening suspected adverse reactions to FDA no later than 7 calendar days after initial receipt of the information.
- Dr. Frank will report any 1) serious, unexpected suspected adverse reactions, (2) findings from other clinical, animal, or in-vitro studies that suggest significant human risk, and (3) a clinically important increase in the rate of a serious suspected adverse reaction to FDA no later than 15 calendar days after determining that the information qualifies for reporting.
- Dr. Frank will submit annual progress reports within 60 days of the anniversary of the date that the IND became active.

26. IMPACT ON STAFF

ACTRI research nursing staff will perform the blood draws and EKG for subjects on the Pre-Scan Study Visit. Each staff member will be trained in phlebotomy and EKG procedures. If the secondary option is chosen for the Pre-Scan Study Visit, staff members at these facilities will be trained in EKG and phlebotomy procedures by their respective facility.

27. CONFLICT OF INTEREST

The PI and key research staff have no conflicts of interest or financial disclosures to declare.

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