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Proposed Plan of Research: Tryptophan, mood and cognition in Anorexia Nervosa

Specific Aims
The neurotransmitter 5-hydroxytryptamine (5-HT), or serotonin, is involved in cognitive processes [1], temperament[2], personality[3] and the development and maintenance of various forms of psychopathology[4, 11]. Basic research in the functional properties of the serotonin system may have far-reaching implications. Numerous studies have examined the role of depressed 5-HT levels on mood and cognition, concluding that 5-HT is critical for behavioral and affective regulation[5]. However, chronically elevated 5-HT levels and implications for affective, cognitive and perceptual functioning are less well understood. The purpose of this study is to determine the functional significance of persistently elevated serotonin on affective, cognitive, perceptual and neuroendocrine processes.

Because individual variations in functioning may not be apparent under basal conditions, differential functioning will be examined by assessing individuals’ responses to acute increases or decreases in serotonin level using dietary manipulations. Two groups will be tested: a group hypothesized to have normal 5-HT levels (healthy controls), and a group hypothesized to have persistently elevated 5-HT levels (a clinical population and unaffected relatives of individuals in the clinical population).

I hypothesize that chronically elevated serotonin heightens anxiety, contributes to perceptual aberrations, and impedes certain cognitive processes. Second, I hypothesize that these effects will be maximally observed under conditions where 5-HT is exogenously elevated.

Background
Tryptophan depletion and loading protocols are established methods of acute, noninvasive manipulation of the serotonin system. These protocols achieve depression or elevation of 5-HT by depleting or augmenting 5-HT’s biological pre-cursor, the amino acid L-tryptophan. Administering an amino acid mixture deficient in tryptophan lowers blood plasma tryptophan levels and the ratio of tryptophan to other large neutral amino acids, as well as levels of 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF)[5]. Administering an amino acid mixture with augmented tryptophan causes increases in blood plasma tryptophan and CSF 5-HIAA[1]. Previous studies utilizing these methods have shown that while tryptophan loading facilitates some aspects of cognition, it can impede working memory[1]. In addition, tryptophan depletion results in affective instability in healthy people and those with histories of affective disturbance [5]. It is more difficult to define a population with chronically high 5-HT levels.

One potential sample consists of individuals with the eating disorder Anorexia Nervosa (AN). AN is a psychiatric disorder characterized by intense fear of becoming fat and refusal to maintain a healthy minimum body weight. Ill AN patients exhibit signs of depressed 5-HT level, likely due to malnutrition. However, 5-HT has been found in elevated levels in recovered anorexics[6]. It is thought that 5-HT levels are also elevated pre-morbid to anorexia in the same manner that perfectionist, obsessional and anxious traits consistent with elevated 5-HT precede AN and persist after recovery[7]. Further, the 5-HT receptor 5-HT2A, suspected to be involved in feeding behavior, has been found in reduced quantities in ill plus recovered AN patients in brain regions of interest, consistent with dysregulated 5-HT [8, 9]. Interestingly, cognitive disturbances are also found in women with anorexia nervosa, independent of co-morbidity with depression[10].

Recently, it was shown that tryptophan depletion reduces anxiety in anorexic and recovered anorexic women to a greater degree than control healthy women[11]. If this finding can be replicated, it suggests that AN women show elevated functioning of the 5-HT system (despite depressed 5-HT level while ill) that is relieved by depletion. Thus, 5-HT dysregulation might play a role in the development or maintenance of AN. More specifically, it has been proposed that AN women reduce anxiogenic elevated 5-HT levels by starving themselves. Eating less reduces the amount of tryptophan ingested, crudely reduces levels of 5-HT, and alleviates anxiety caused by excessive 5-HT[11].

Any study examining neurobiological variables in active AN patients must take into account the confounding effects of malnutrition. This is often dealt with by including recovered AN patients in studies along with active patients; however there may be more lasting biological consequences induced by having AN. One creative solution to this dilemma is to capitalize on the estimated high genetic component of AN. From twin studies, anorexia is estimated to have a heritability between 0.5 and 0.8 as determined from mono- and dizygotic twin concordance rates[12]. Thus, to control for physiological changes associated with malnutrition in anorexia nervosa, I plan to study the effects of tryptophan depletion and augmentation in unaffected first-degree female relatives of anorexic women, as well as in anorexic women and control women.

Research design and Methods
In a double-blind, within-subjects, counterbalanced design, I will temporarily and on separate occasions increase and decrease levels of 5-HT via tryptophan depletion and tryptophan augmentation in anorexic women, unaffected first-degree female relatives of anorexic women, and control women with no personal or family history of anorexia. Women in each of the three groups will be administered amino acid beverages in each of three conditions: one mixture will be deficient in tryptophan, one will be augmented with tryptophan, and one will be a balanced control amino acid mixture. The nature of which amino acid mixture is ingested on each day will not be known to the participant or the researcher who performs the testing. Participants will complete mood and anxiety level measures hourly – particularly the Profiles of Mood States, which has been shown to be reliably sensitive to subclinical changes in
mood. Blood samples will be collected at baseline and five hours after amino acid consumption. These samples will be analyzed for tryptophan levels, as well as prolactin and cortisol to determine neuroendocrine response to changes in 5-HT levels. Participants will complete cognitive tests of working memory and body image perception using a standard task.

**Hypotheses**

I hypothesize that tryptophan depletion will decrease anxiety in AN patients and unaffected female relatives to a greater extent than control women. Tryptophan loading will increase anxiety in AN patients and unaffected female relatives to a greater extent than control women, due to hypothetically pre-existing elevated level of 5-HT. 5-HT manipulation will moderate responses to the body image affective perception task in all groups. In all groups, tryptophan depletion will facilitate working memory processes. Tryptophan augmentation will impair these processes. I would expect these results to be exaggerated in AN patients and unaffected female relatives compared to controls. AN patients and unaffected female relatives are hypothesized to show more blunted neuroendocrine responses than control women to 5-HT manipulations.

**Statistical Analyses**

Repeated measures ANOVA’s will be performed to determine condition by group interactions. This is an area of training in which I hope to gain expertise through coursework in statistics and more in depth data analytic experience.

**Originality and authorship of project**

I am the principle investigator in this project, which I am developing in collaboration with Monica Luciana, Ph.D. and Scott Crow, M.D. for my dissertation research. My interest in developing a project of this nature stems from several undergraduate and post-baccalaureate experiences. I first became interested in the tryptophan depletion paradigm as a means to study 5-HT from reading Walter Kaye and colleagues’ work for a Biological Bases of Eating Disorders and Obesity seminar when I was a student at Florida State University. My interest in basic serotonin research was spurred by my work on the 5-HT transporter polymorphism (5-HTTLPR).

**Reasons for selection of Graduate Institution**

The University of Minnesota is an appropriate graduate school for me primarily for two reasons: first, the quality of the research match, and second, the quality of available resources and educational opportunities necessary to my conduct of the proposed research. The research match is excellent – my research mentor, Monica Luciana, has conducted several pharmacological challenge studies using methods similar to those proposed. Further, Scott Crow, M.D., has conducted 5-HT-related drug studies and is an internationally prominent researcher in eating disorders. Resources necessary to conduct this research are available. Several area eating disorder treatment centers provide access to clinical populations needed for the proposed study. The General Clinical Research Center at the University of Minnesota provides nursing and nutritional services in a medical setting for research projects. Also, the Minnesota Obesity Center provides financial and logistical support to research involving eating behaviors.

**References**

2. Auerbach, J., et al., *Dopamine D4 receptor (D4DR) and serotonin transporter promoter (5-HTTLPR) polymorphisms in the determination of temperament in 2-month-old infants.* Molecular Psychiatry, 1999. 4: p. 369-373.