1. Title Page

Protocol Title: Fluoxetine's effects on attention and emotional memory in anxious and depressed youth and adults.

Protocol Number: 01-M-0192
Date of This Submission/Version: May 11th, 2023

Principal Investigator

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Total requested accrual

Subjects of Study	Total to be recruited	Total to be studied	Sex	Age Range
Children and adolescents with an anxiety disorder	600	150	M & F	8-17
Children and adolescents with major depression	80	60	M & F	8-17
Psychiatrically healthy children and adolescents	750	150	M & F	8-17
Adults with major depression	150	100	M & F	18-65
Adults with an anxiety disorder	200	60	M & F	18-65
Psychiatrically healthy adults	750	150	M & F	18-65
Total participants in study	2530	670		

Project Uses Ionizing Radiation: ☒ No ☐ Yes (attach *RSC/RDSC documentation)*
- ☐ Medically-indicated only
- ☐ Research-related only
- ☐ Both

IND/IDE ☐No ☒Yes *(attach FDA documentation)*
Drug/Device/#__NSR_____
Sponsor: ____NIMH_____

Durable Power of Attorney ☒ No ☐ Yes
Multi-institutional Project ☒ No ☐Yes

Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☒ No ☐ Yes
Agreement type and number _____Expiration Date_____

Confidential Disclosure Agreement ☒ No ☐ Yes

Samples are being stored ☐ No ☒ Yes

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3. Precis

Objective: This protocol uses functional magnetic resonance imaging (fMRI) and magneto- and electro-encephalogram (M/EEG) to examine neuro-cognitive correlates of pediatric and adult mood and anxiety disorders. The primary goal of the project is to document, in pediatric anxiety disorders and major depression, perturbations in brain systems mediating attention biases, fear conditioning, emotional memory, and response to various forms of motivational stimuli. As one secondary goal, the project measures the relationship between these factors and treatment response to either fluoxetine, a specific serotonin reuptake inhibitor (SSRI), or cognitive behavioral therapy (CBT). Another secondary goal examines similar associations in adults.

Study Population: A total of 2530 children, adolescents, and adults will be recruited. Most subjects will not be able to complete all procedures. We seek to comprehensively study 150 juveniles with only a current anxiety disorder, 60 juveniles with current major depression, 150 juveniles with no psychiatric disorder, 100 adults with major depression, 60 adults with an anxiety disorder, and 150 adults with no psychiatric disorder. To achieve this, we are recruiting 2530 individuals.

Design: Subjects will be tested using fMRI and M/EEG paradigms designed to examine brain regions engaged when processing motivationally salient stimuli, as assessed during attention, memory, social interaction, reward, and fear-conditioning paradigms. After these initial fMRI and M/EEG tests, subjects with depression or an anxiety disorder receive treatment. Treatment will comprise open treatment with either fluoxetine or CBT, augmented with computer-based attention retraining, delivered in a randomized-controlled design, with random assignment to either active or placebo attention-training regimens. Adolescent subjects then will be re-tested after eight-weeks using only the attention, memory, and conditioning paradigms.

Outcome Measures: Prior imaging studies note that tasks requiring attention modulation, emotional memory, social interchange, and fear conditioning engage brain regions previously implicated in adult mood and anxiety disorders. These regions include most consistently the amygdala and ventral prefrontal cortex. Moreover, imaging studies of reward function implicate the striatum and prefrontal cortex in adult mood disorders. ***As a result, we hypothesize that attention, memory, social interaction, reward, and conditioning paradigms will engage the amygdala, ventral prefrontal cortex and striatum in both psychiatrically healthy and impaired subjects. Moreover, we hypothesize that these healthy and psychiatrically impaired groups will differ in the degree of engagement.***

Juvenile subjects also will be treated for eight-weeks, and a subset will be re-tested with fMRI and M/EEG. ***We predict that pre-treatment abnormalities in neural circuitry will predict response to treatment, such that increased amygdala and prefrontal activation will occur in individuals who show the strongest response to treatment. Moreover, we hypothesize that effective treatment will normalize abnormalities in attention and emotional memory, as manifest in fMRI and M/EEG measures.***

4. Introduction/Scientific Rationale

Three areas of research inform this protocol - - research on: i) associations among pediatric and adult mood and anxiety disorders;[1-11] ii) neuro-cognitive correlates of adult mood and anxiety disorders;[12-33] as well as iii) neuro-cognitive correlates and treatment of pediatric mood and anxiety disorders.[34-47]

4.1. ***Associations among pediatric and adult mood/anxiety disorders***

4.1.1. Comorbidity: Most children with an anxiety disorder suffer from multiple disorders[1, 4, 5, 7-9]. Comorbidity is particularly strong among social phobia, separation anxiety, and generalized anxiety disorders.[4] Such comorbidity complicates studies on risk factors and treatments. As a result, most current treatment and risk factor research on childhood anxiety disorders groups together the diagnoses of social phobia, separation anxiety disorder, and generalized anxiety disorders.[1, 3-5, 7-9, 48] In fact, virtually all treatment trials combine the three diagnoses into a single category.[5, 48] The current protocol will combine these three conditions into one anxiety group. There also is considerable comorbidity between childhood anxiety and depressive disorders. This further complicates studies on disorder-specific correlates. The current protocol compares healthy youth with two patient groups: i) youth with any one of three anxiety disorders (social phobia, separation anxiety disorder, and generalized anxiety disorder) in the absence of depression; and ii) youth with depression, with or without anxiety. Sub-analyses also will contrast depressed subjects with and without anxiety disorders and subjects with specific anxiety disorders.

4.1.2. Associations Between Disorders in Children and Adults: Youth with mood or anxiety disorders face a two-to-five fold increased risk for adult depressive and anxiety disorders.[1-11] In fact, most adults with a mood or anxiety disorder had shown previous signs of a mood or anxiety disorder during childhood.[1, 4, 5, 8] Youth with various mood and anxiety disorders also show high familial loading for mood and anxiety disorders.[2, 6, 10, 11] Parental history of a mood or anxiety disorder may represent the most potent risk factors for personal history of a disorder in a child.[7] Based on such data, recent studies on pathophysiology of adult mood and anxiety disorders provide insights on pathophysiology in pediatric conditions.

4.2. ***Cognitive processes and brain circuitry in adults:*** Mood and anxiety disorders are associated with cognitive abnormalities that may result from disruptions in neural circuits regulating attention and memory.

4.2.1. Attention: A wealth of research documents enhanced attention to threat in adult anxiety disorders. Both basic and clinical studies elucidate factors that produce such effects. Adult anxiety disorders involve enhanced attention bias for threat cues.[19-21, 23-27, 29, 32, 49] Attention bias is demonstrated in various tasks, including interference[9, 28, 32, 47, 49] and orienting tasks.[9, 28, 47, 49] In general, orienting tasks better isolate brain circuits involved in attention modulation.[27, 28]As a result, the current protocol relies on orienting tasks. While enhanced attention bias is most consistently found in anxiety, effects emerge with less consistency in depression.[19, 27, 28] Interestingly, the time course of bias differs in anxiety and depression. Bias in anxiety but not depression occurs with short presentation of emotional cue stimuli (17-33 msec), whereas enhanced attention bias in both anxiety and depression may occur with longer presentation of emotional cue stimuli (200-750 msec).[19, 27, 28]As in Mogg and Bradley,[19, 27, 28]the current protocol elicits attention bias by presenting emotionally evocative facial cue stimuli at both long and short exposures.

Because attention bias can be reliably generated in animals through fear conditioning experiments, common brain structures are thought to regulate aspects of attention bias and fear conditioning.[24, 25, 50-52] This includes the amygdala and ventral prefrontal cortex. Imaging studies in humans note the engagement of these structures during attention bias paradigms.[53-56] For example, angry faces that affect attention in orientation paradigms consistently engage ventral aspects of the prefrontal cortex, a region strongly implicated in mood and anxiety disorders.[53, 55-57]

In humans, effective treatments normalize attention bias associated with anxiety.[25, 27, 32] In animals, anxiolytics reduces attention bias for fear-related stimuli.[58-60] Neuroimaging studies suggest that medications

could exert such effects by altering functioning in the amygdala and ventral prefrontal cortex.[61, 62] Finally, most studies of fear conditioning and attention rely on relatively simple paradigms with questionable ecological validity. Considerable recent interest has emerged in adapting reward or social-interaction paradigms to provide attention-related tasks with stronger ecological validity. Moreover, these tasks also rely on a range of aversive stimuli to evoke attention. This has raised major questions on the degree to which enhanced attention responding to threat represents enhanced sensitivity to a relatively narrow or broader range of threatening stimuli, as occurs across anxiety disorders or to unique subsets of patients.

[111-115]Currently, most research in this area relies on words or pictures. However, abnormal attention to physiological perturbations generally manifests with larger effect sizes than abnormal attention to words or pictures. More work is needed that compares responses among the various picture-based and physiologic paradigms. [112] This includes studies attempting to replicate findings on abnormal responding to social stimuli in social anxiety disorder, as opposed to abnormal responding to respiratory stimuli in separation anxiety disorder. Such disorder-general and disorder-specific responses can be contrasted with the dot-probe task, fear-conditioning paradigms, and CO2 inhalation, a paradigm eliciting particularly large, consistent, and relatively specific between-group differences in research on separation anxiety disorder.

In work on attention, paradigms often attempt to elicit anxiety-related attention problems that arise under the stress of a cognitive task. This interest in stress-evoked attention perturbations reflects a view that hypersensitivity to stress is an important characteristic of anxiety disorders. Beyond assessing anxiety symptoms, this protocol also uses clinical measures to assess the sensitivity of children and adolescents to stressful events, which can also impact performance on the paradigms used in the protocol. The COVID-19 pandemic of 2020 enhanced the level of stress that children enrolling in this protocol will experience. As a result, the protocol will also assess sensitivity to this unique form of stress and will obtain additional follow-up data from participants. This will provide data on brain function and clinical outcomes in children and adolescents as it relates to a wide range of stressful events, including those that are closely related to the pandemic. While the current study tests no hypotheses specifically related to the stress of the pandemic, the unique circumstances of the pandemic create opportunities for enhanced stress exposures that need to be quantified. This is necessary to fully capture relationships between stress exposure and both brain function as well as treatment response, the two main targets related to anxiety symptoms in the current protocol.

4.2.2. Memory: Considerable research implicates memory abnormalities in both mood and anxiety disorders. Both basic science and clinical studies elucidate factors contributing to these associations.

Mnemonic abnormalities are found in adults with major depression, panic disorder, and social phobia.[13-16, 20, 23, 24, 30, 31, 33] Such patients tend to show both an absolute decrement in declarative memory[33] as well as a recall bias for emotionally-negative material, termed “emotional memory bias”.[14, 15, 31] While this bias has been demonstrated using both evocative words and pictures, evocative words pose problems for child-based studies, given developmental changes in abstract thought. As a result, the current study uses pictures to probe both general memory as well as emotional memory. Prior studies in adults have used both standard facial expressions of emotion[22] as well as standard slides from the International Affective Picture Series (IAPS).[21] Both sets of stimuli will be used in the current study. As in research on attention, research considers emotional memory to relate closely to stress hypersensitivity. As a result, data on emotional memory also quantifies brain function related to stress, including pandemic-related stress.

Considerable research delineates cortico-limbic circuits involved in emotional modulation of memory. Studies in rodents implicate the amygdala and prefrontal cortex,[61-63] while neuroimaging studies in adults implicate analogous structures in human emotional memory.[64] These imaging studies rely on face encoding or IAPS rating paradigms that will be employed in the current study.

Studies in both animals and adults suggest that effective treatment for mood and anxiety disorders alters functioning in brain circuits that regulate memory.[20, 21, 23, 24, 26, 30, 61] Specifically, antidepressants reduce memory bias. These effects are thought to derive at least partially through moderation of activity in a circuit encompassing the basolateral amygdala, hippocampus, and prefrontal cortex.

4.3. ***Cognitive Processes and treatment in adolescents:***

4.3.1. Cognition: While the evidence is less definitive, adolescents with mood and anxiety disorders, much like adults, also exhibit attention and memory bias. [35, 40-43, 45-47, 65-68] However, since most children with depression exhibit signs of anxiety, it is difficult to draw conclusions on the nature of specific association between attention bias and pediatric depression. Taken together, the available literature finds signs of developmental continuity in the cognitive correlates of mood and anxiety disorders. Moreover, recent brain imaging studies,[69] including preliminary studies emanating from the current protocol,[65-68, 70] demonstrate perturbations in amygdala-prefrontal cortical circuitry in pediatric depressive and anxiety disorders.

4.3.2. Treatment: SSRIs are established treatments for adult depression and anxiety. Existing data suggest similar effects in youth.[34, 36-39, 48] Two SSRIs possess FDA indications for pediatric obsessive-compulsive disorder (OCD).[38] Available data also document strong benefits in anxiety.[36, 48] In other work, CBT shows promise in pediatric anxiety disorders and major depression. Nevertheless, due to the paucity of head-to-head trials, it remains unclear the degree to which specific treatments provide particular benefits for specific pediatric mental disorders. Recent work also suggests that the training of attention through cognitive remediation strategies may augment response of anxiety disorders to SSRIs or CBT. Thus, the current protocol also will implement attention retraining as part of treatment with SSRIs or CBT.

5. Study Objectives or Hypotheses:

The primary goal of the project is to document in pediatric mood and anxiety disorders perturbations in brain systems mediating attention, memory, and response to various motivational stimuli. As one secondary goal, the protocol also will examine the relationship between brain activation prior to treatment and response to treatment over eight weeks as well as changes in fMRI and M/EEG activation before and after treatment. This goal is considered secondary given the paucity of knowledge concerning both aspects of treatment as well as aspects of brain function in pediatric mood and anxiety disorders. In anxiety disorders, as an additional secondary goal, preliminary data will be gathered on the utility of computer-based training on cognitive tasks designed to increase response to CBT.

Finally, data also will be acquired in adults to test the hypothesis that pediatric mood and anxiety disorders exhibit similar perturbations as found in adult mood and anxiety disorders. This prediction is based on available data in separate studies of adolescents and adults that document parallels in the correlates of mood and anxiety disorders across development. These data also suggest that distinct cognitive factors distinguish anxious and depressed from healthy individuals. Namely, because attention bias more strongly relates to anxiety than depression, we hypothesize that brain circuits associated with enhanced attention bias can be differentially engaged among anxious and depressed youth and adults, with anxious groups appearing most different from healthy ones at both ages. Conversely, because memory bias more strongly relates to depression than anxiety, we hypothesize that brain circuits associated with memory bias can be differentially engaged among depressed and anxious groups, with the depressed groups appearing most different from healthy groups. We hypothesized that both anxiety and depression are associated with abnormal neural responses on social interaction fMRI and M/EEG tasks.

5.1. ***Baseline Dysfunction in Disorders***: Our primary goal is to compare functioning in the amygdala, ventral prefrontal cortex, and striatum in healthy and anxious or depressed youth. A secondary goal extends this work to adults. Prior studies suggest that mood and anxiety disorders are associated with deficits in attention, emotional memory, reward processing, and social interaction. Imaging studies in adults suggest that these deficits result from dysfunction in the amygdala and ventral prefrontal cortex. The current study will use fMRI and M/EEG to examine brain regions engaged by attention, emotional memory, reward-

processing, social interaction, and fear-conditioning paradigms in pediatric and adult depression and anxiety disorders. As noted above, these paradigms are designed to quantify aspects of brain function related to stress sensitivity and exposure. As a result, levels of stress sensitivity and exposure will also be quantified to explore the degree to which they mediator or moderate associations between brain function and pediatric anxiety or depression.

We hypothesize that fMRI- and M/EEG-measured attention bias, emotional memory, social interaction, fear conditioning, and reward paradigms will engage cortico-limbic brain regions in both healthy and impaired subjects, both among youths and among adults. However, healthy and impaired groups will differ in the degree of amygdala, prefrontal, and striatal engagement.

For attention bias and fear conditioning paradigms, this difference is expected to be most prominent in anxiety. Specifically, we hypothesize that adolescents and adults with anxiety disorders will exhibit enhanced amygdala and ventral prefrontal cortex activation when attending to threat cues. This enhanced activation will occur both relative to healthy individuals and relative to individuals with major depression.

In adolescent and adult major depression, in contrast, we hypothesize that enhanced activation will occur during an emotional memory paradigm, manifest as greater amygdala and prefrontal cortex activation in successfully vs. unsuccessfully remembered emotional stimuli. This enhanced activation will occur both relative to healthy groups and relative to anxiety-disorder groups.

For social interaction tasks, we expect to observe similar differences in anxiety and depression. Thus, both patient groups are hypothesized to differ from healthy subjects but not from each other.

Finally, for reward tasks, we expect to observe reduced neural responses to reward stimuli and enhanced response to punishment stimuli in depression. Anxiety would be marked by enhanced response to both negative and positive incentives.

5.2. ***Treatment:*** Effective treatment ameliorates neuro-cognitive correlates of adult mood and anxiety disorders.[21, 23-26] In children, studies document benefits of SSRIs and CBT but none examine the relationship between treatment and brain function, a secondary focus of the current study. These measures of brain function quantify aspects of stress sensitivity. As a result, levels of stress sensitivity also will be quantified to explore the degree to which they relate to anxiety treatment outcome and its relationship with brain function mediating the persistence and development of anxiety.

We hypothesize that subjects who respond to treatment will differ from subjects who do not, based on their pattern of behavior and fMRI and M/EEG activation pre-treatment. Specifically, we predict that responders and non-responders will differ in their pattern of attention bias, memory performance, and amygdala/prefrontal cortical activation.

We also hypothesize that effective treatment will reduce attention-related amygdala and prefrontal cortex dysfunction in adolescents with anxiety disorders. Of note, this hypothesis will be tested only in youth; adults will not be re-tested after treatment, nor will analyses compare adults and adolescents after treatment. Specifically, we hypothesize that anxiety-disorder subjects who respond to treatment, relative to those who do not respond, will differ in fMRI and M/EEG amygdala and ventral prefrontal activations when attending to threat, such that only non-responders will continue to differ from healthy adolescents. In depression, we expect successful treatment to normalize abnormalities in amygdala and prefrontal activation in an emotional memory paradigm.

The project will test these hypotheses by studying three groups of youths: i) psychiatrically healthy youth ["healthy"]; ii) youth without major depression but with social phobia, separation anxiety, or generalized anxiety disorders ["anxious"]; iii) youth with major depression, with or without social phobia, separation

anxiety, generalized anxiety disorders ["depressed"]. In addition, to document commonalities and differences with adults, we will study an additional three groups: iv) adults with an anxiety disorder ["anxious']; v) psychiatrically healthy adults ["healthy"]; vi) adults with major depression.

Prior to treatment, subjects will undergo fMRI and M/EEG scanning while performing paradigms hypothesized to differentiate relevant groups. Subjects with mood and anxiety disorders will then be treated for eight weeks. Adolescent patients will be re-studied using the fMRI and M/EEG attention and emotional memory paradigms. In all subjects, data for social interaction and reward tasks only will be acquired once, prior to treatment.

6. Subjects

6.1. ***Description of Study Populations:***

A total of 360 juvenile subjects will be studied comprehensively, comprising groups of "healthy," "anxious," and "depressed" youth. Of note, we anticipate enrolling far more than 360 subjects (see accrual numbers; total accrual ceiling is set to be n=2530), as not all subjects enter all phases of the study. For example, some subjects may remit during the initial pre-treatment phases of the study. Such subjects will, of course, not receive treatment. Other subjects may only tolerate a portion of the procedures proposed here. By "studied comprehensively", we mean that we plan to obtain data for *each* key assessment measure in a total of 360 juveniles, as stratified below.

Based on prior studies, 80% of the depressed subjects are expected to meet criteria for a current anxiety disorder. Thus, our goal is to collect data for each measure among 20 depressed adolescents without an anxiety disorder as well as 40 depressed adolescents with a comorbid anxiety disorder. In terms of anxiety disorders, we expect to study on each of our tasks approximately 80 subjects with social phobia, 40 subjects with separation anxiety disorder, and 80 subjects with generalized anxiety disorder. These numbers exceed n=150 due to comorbidities. Of note, we plan to directly contrast specific anxiety disorders, particularly generalized and social anxiety disorders. Thus, a relatively large sample size is needed.

Hypotheses for studies in adult mood and anxiety disorders are identical to those in youth. Moreover, we expect comparable effect sizes for between-group comparisons of healthy and impaired adults as found in healthy and impaired adolescents. As a result, we will study 60 adults with anxiety disorders, of whom approximately 30 will have social phobia; 30 will have generalized anxiety disorder; and 20 will have panic disorder. This will provide sufficient data for power analyses in specific anxiety disorders.

The rationale for sample sizes follow from prior treatment and neuropsychological studies (see power analysis section). The current project is powered to examine the neurobiology of each task in both pediatric mood and anxiety disorders, considered individually. This represents the main goal of the study. Given heterogeneity in treatment outcome, it is not feasible to study sufficient numbers of subjects to provide definitive insights on prediction of treatment outcome. Thus, the study is not designed, as a primary goal, to examine prediction of response to one or another treatment.

In addition to these 650 juveniles, 650 psychiatrically healthy adult volunteers will be studied using similar procedures to those used among the psychiatrically healthy juveniles. Finally, 100 adults with major depression and 60 adults with an ongoing anxiety disorder (panic disorder, social phobia, or generalized anxiety disorder) will be studied using these methods. These 120 adults will undergo the procedures described in this study. Adults with major depression or anxiety will be treated with fluoxetine by a member

of Dr. Pine's staff. Studies in adults will not acquire data after treatment. Hypotheses and statistical power considerations are identical in studies of adults and youths.

As of June 3, 2011, 1196 subjects have been accrued into the protocol with a ceiling of 2530 subjects. This includes 1141 subjects who are study completers.

6.2. *Inclusion Criteria:*

INCLUSION CRITERION	COMMENTS
ALL JUVENILE SUBJECTS	
Age: 8-17 (subjects who consent as 17-year-olds but turn 18 during the course of the study will be eligible to complete all procedures completed by other subjects who consent as 17-year-olds but do not turn 18).	
Consent: can give consent/assent	Parents will provide consent; minors will provide assent
IQ: all subjects will have IQ>70	Assessment relies on either a WASI or assessment by trained clinical staff during the subject's screening visit. Completion of required activities during the screening visit requires an IQ above 70.
Language: all subjects will speak English	Tasks in this protocol have not been validated in languages other than English
ALL ADULT SUBJECTS	
Age: 18-65	
Consent: can give consent	
IQ: all subjects will have IQ>70	Assessment relies on either a WASI or assessment by trained clinical staff during the subject's screening visit. Completion of required activities during the screening visit requires an IQ above 70.
Language: all subjects will speak English	Tasks in this protocol have not been validated in languages other than English
ALL SUBJECTS WITH AN ANXIETY DISORDER	
Diagnosis: Current Diagnosis of Social Phobia, Separation Anxiety, Generalized Anxiety Disorder, or Panic Disorder	Based on K-SADS (juveniles) or SCID (adults)
Symptom Severity: Clinically significant, ongoing anxiety symptoms	This will be documented by clinician review with patients and their families during at least two visits with families.
Clinical Impairment: Clinically significant, ongoing distress or impairment from anxiety	This will be documented by clinician review with patients and their families during at least two visits with families.
ALL SUBJECTS WITH A MOOD DISORDER	
Diagnosis: Current Diagnosis of Major Depression	Based on K-SADS (juveniles) or SCID (adults)
Clinical Impairment: Clinically significant, ongoing distress or impairment from depressive symptoms	This will be documented by clinician review with patients and their families during at least two visits with families.
Symptom Severity: Clinically significant, ongoing depressive symptoms	This will be documented by clinician review with patients and their families during at least two visits with families.
ALL PREVIOUSLY ENROLLED ADOLESCENT PATIENTS, CHILD AND ADULT HEALTHY VOLUNTEERS, AND ALL HEALTHY VOLUNTEERS TURNED PATIENTS	
Diagnosis: Current Diagnosis of Social Phobia, Separation Anxiety, Generalized Anxiety Disorder, or Panic Disorder; No current diagnosis	Based on K-SADS (juveniles) or SCID (adults)

Clinical Impairment (as applicable): Clinically significant, ongoing symptoms	This will be documented by clinician review with patients and their families during at least two visits with families.
Symptom Severity (as applicable): Clinically significant, ongoing symptoms	This will be documented by clinician review with patients and their families during at least two visits with families.

6.3. ***Exclusion Criteria:***

EXCLUSION CRITERION	COMMENTS
ALL SUBJECTS	
1. Any serious medical condition or condition that interferes with fMRI or M/EEG scanning, and for patients electing medication, any condition that increases risk of SSRI treatment.	All patients will complete a medical history. Healthy volunteer participants will be medication- free and have no current serious medical conditions, based on a review of their medical history.
2. Pregnancy	
3. Current use of **any** psychoactive substance; current suicidal ideation; current diagnosis of attention deficit hyperactivity disorder (ADHD) of sufficient severity to require pharmacotherapy	These factors could complicate treatment with an SSRI. No subject on medication will be accepted into the trial. Subjects will not be taken off of medications to enter the trial.
4. Current diagnoses Tourette's Disorder, OCD, post-traumatic distress disorder, conduct disorder	These factors may be effected by SSRI treatment, influencing ability to detect effects on anxiety/depression
5. Past or current history of mania, psychosis, or severe pervasive developmental disorder	Same as above
6. Recent use of an SSRI; all subjects must have been free of any SSRI-use for at least one month (fluoxetine six months) and must not have been treated with an SSRI for their current depressive episode.	This is designed to exclude subjects who have failed a trial of an SSRI for their current episode of major depression. For previously enrolled participants, including patients and healthy volunteers, current use of an SSRI does not exclude participation from follow-up research tasks.
7. NIMH employees and staff and their immediate family members will be excluded from the study per NIMH policy.	
HEALTHY ADULT SUBJECTS	
1. Any current psychiatric diagnosis	Assessment relies on SCID

7. Study Design and Methods:

7.1. Study Overview:

The study has four phases: i) recruitment, assessment, and training; ii) pre-treatment MRI and M/EEG scanning; iii) treatment; iv) post-treatment MRI and M/EEG scanning. Recruitment, screening, and training require three visits. Subjects will be screened on an initial visit. On a second and third visit, subjects will undergo training in an MRI simulator as well as neuropsychological testing. MRI and M/EEG sessions are each designed to last one hour but may extend longer. No scanning day will extend beyond 120 minutes, as we have found that subjects become fatigued if longer scanning sessions are required. If scanner time is not available for a full initial session or if subjects prefer for any reason, the pre-treatment procedures may be divided. The treatment protocol relies on established procedures. Pre and post-treatment scanning procedures are identical for three tasks, though different pictorial stimuli will be used in the two sessions to maintain a consistent level of novelty. Other tasks are only given pre-treatment.

7.2. Recruitment:

All subjects will be screened through a screening protocol, NIMH-01-M-0254, *The evaluation of patients with mood and anxiety disorders and healthy volunteers*. Of note, specific procedures will be used to

facilitate recruitment of each subject group. For psychiatrically healthy participants, volunteers will be solicited using advertisements and direct mail postcards. The direct mail postcards will be sent, with a disclaimer, to local households identified as having children 8-17 years old. Subjects with mood and anxiety disorders will be recruited when they seek treatment through the Intramural Research Program at NIMH. We will work to make the community aware of ongoing treatment studies through various endeavors. We will send direct mail postcards, with a disclaimer, to local households identified as having children 8-17 years old, as well as display web advertisements to recruit children with anxiety or depression. Advertisements will be displayed on the Washington Parent Magazine website (http://www.washingtonparent.com) as an online banner/sidebar ad that will link to the NIMH Patient Info Pediatric Anxiety Study webpage (http://patientinfo.nimh.nih.gov/AnxietyDisordersPediatric.aspx#116). This webpage is used for the recruitment of subjects with anxiety disorders and discusses the study (01-M-0192) in more detail. We will use advertisements as needed in other settings, such as the print media. In addition, we will notify attendees at various presentations that we give in and around the NIH community of available ongoing treatment studies by making flyers available at the presentation, with the goal of making the community aware of ongoing treatment studies. These procedures have been approved by Susanna Sung.

Finally, subjects may be referred from other institutions. This will include healthy adults. Currently Dr. Nathan Fox of the University of Maryland (UMD) is a PI on a study at his institution. Healthy adults may be referred to NIH 01-M-0192 based on information discovered during participation in those studies. If a potentially qualified individual is found, Dr. Fox will provide contact information to the individual who will initiate first contact if interested. Dr. Fox will not know if an individual participates or not unless the individual chooses to tell them. No data will be shared between the studies at UMD or NIH. The individual study at UMD will be approved by the IRB at their institution, including referrals to the NIH 01-M-0192. Similarly, this also will include healthy adolescents or adolescent patients who meet inclusion criteria. Dr. Roxann Roberson-Nay from VCU has been a collaborator on the current protocol, since the time that she received post-doctoral training at the NIMH-IRP. She is initiating a recently-funded study in Northern Virginia, in close proximity to the NIH. This extramurally-funded study (R01-MH098055) includes Dr. Pine as a collaborator, and the study uses similar neuropsychological measures that are employed in the current protocol. If a potentially qualified individual is found, Dr. Roberson-Nay will provide contact information to the family of this individual who will initiate first contact if interested. Unlike with Dr. Fox, however, Dr. Roberson-Nay already is approved to receive data collected as part of the current protocol, based on her long-standing collaborative role in the protocol.

NIH employees and staff will not be directly recruited by or through their supervisors or co-workers to participate in this study.

7.3. Screening Methods:

All subjects will be screened through a screening protocol, NIMH-01-M-0254, *The evaluation of patients with mood and anxiety disorders and healthy volunteers*.

7.4. Study Design:

The overall study design includes two sets of projects. One set uses a case-control design, where various patient and healthy groups are compared, based on various parameters. The second set uses a randomized-controlled trial design, where two treatments are compared.

Of note, participants in this protocol may be referred for participation in a protocol at the University of Maryland (UMD Protocol 01-0115; An Investigation of Infant Temperament; PI: Nathan Fox). For subjects who chose to participate in both studies, procedures common to both protocols will only be done once and may be done either at NIH as part of this protocol or at UMD as part of Protocol 01-0115. The data from these procedures will be shared between the two institutions to avoid duplicate testing.

7.5. Study Procedures:

All subjects are expected to complete all four phases of this study within six months, though precise timing depends on the needs of the families. Typically, initial recruitment, consent, and the pre-treatment assessments are completed within three weeks. This is followed by all baseline assessments, usually during the next ensuing three weeks, which are then followed by treatment. Patients will be offered treatment, and they may receive it. For those patients who elect to initiate treatment, patients may have between three and 12 visits as part of the treatment research component of the study. Subjects may receive additional treatment visits, as they are transitioned to the community. All post-treatment procedures and transition into the community typically require three weeks.

All procedures in each of these four phases generate important research data centrally related to the protocol objectives. As a result, all such procedures are centrally related to the research mission of the protocol. In terms of clinical care, the current protocol also examines predictors of treatment outcome, as part of the research. As a result, treatment administered in the main phases of the protocol serves both a research and clinic-care purpose. The treatments are explicitly designed to provide clinical benefits to participants.

Finally, following the completion of research-related treatment, some patients require additional services so that they can be successfully transitioned from the research protocol into the community. These procedures typically take a few weeks to complete, but they may take longer. Every effort is made to provide these services in as efficient a format as possible, so that patients are rapidly transitioned into the community. However, delays can occur due to the limited availability of clinicians in the community and the recognition that research participants deserve clinically-appropriate care. This final set of procedures is not related to research; it is related only to clinical care.

For some of the measures used in the current study, we will compare findings in patients seen at NIH with findings in patients seen at collaborating institutions: University of Wisconsin and Vanderbilt University. This will support the collaboration involving the NIMH, University of Wisconsin and Vanderbilt University. Identical measures will be used across the three study sites. Nevertheless, despite the use of identical procedures, differences in the MRI scanners at the three sites still could contribute to any observed differences in the findings across the three centers. We will use standard procedures to evaluate reliability[103] These procedures require the regular study of the same individual across sites and over time. We only will study healthy adults as part of the ongoing reliability evaluations, each performed across the three sites. Subjects to be studied at the three sites first will be enrolled in the current, NIH study protocol. For subjects who are willing to participate in the reliability study, we will then provide transportation for these subjects so that they can be studied at both Wisconsin and Vanderbilt. Once subjects have been enrolled in the current protocol and have expressed interest in participating in the studies at Wisconsin and Vanderbilt, the teams of Dr. Kalin and Dr. Blackford will be contacted at Wisconsin and Vanderbilt, to provide the name of the interested subject to the collaborating teams. Drs. Kalin and Blackford's teams then will work with the research participant to arrange the timing of the study at their institution. In both instances, travel then will be arranged by the NIH team. Subjects will travel to the MRI centers at Wisconsin and Vanderbilt, where they will be consented into corresponding protocols at each

institution using consent forms approved there and enrolled into the parallel research protocols at these institutions.

For each of the two reliability studies, we hope to study 40 healthy adults on at least two occasions over a two year period, depending on subject availability and the ongoing success of our multi-site efforts. As noted above, these healthy adult subjects will enroll in the respective IRB protocols at Wisconsin, Vanderbilt. For one of the multi-site reliability studies, Wisconsin will serve as the data-coordinating site for the multi-site aspect of the study. For the other study, University of Maryland, as supervised by Dr. Nathan Fox, will serve as the data-coordinating site. Order of participation will be randomized, such that some subjects will be studied first at each of the three sites.

7.5.1. ***Pre-Study Assessment in Clinic and Laboratory:*** These procedures establish subject eligibility and comprehensively characterize subjects before they undergo fMRI or M/EEG. Data acquired during fMRI and M/EEG represent the primary outcome measures.

Subjects will be assessed using four clinical procedures as well as a series of neuropsychological and psychophysiological tests. These procedures will be completed over three visits, though there will be some flexibility in this schedule to accommodate families.

7.5.1.1. *Psychiatric History* (required)*:* All subjects will be screened for lifetime history of psychiatric disorders using the K-SADS Interview,[63] administered by a trained clinician (at least Masters level) supervised by Dr. Pine. Families will also be assessed for family history of psychopathology, through an interview with parents. These interviews may be videotaped. The tapes will be confidential and used only for research purposes, such as making sure that the members of the research team agree on the presence or absence of the diagnoses. In adults, the SCID Interview will be used instead of the K-SADS. The Development and Well-Being Assessment (DAWBA) has been extensively described[128, 129] and we will use it here so that we can compare our data with data from other studies using the DAWBA. It either will be completed on a computer or by a research assistant with the patient and a parent.

7.5.1.2. *Medical History* (required)*:* Participants will receive a comprehensive review of their medical history using the standard Clinical Center Self Report of Medical History form (NIH-532-3). A physical examination performed by a licensed physician or physician's assistant, only if the participant is enrolled as a child patient and is on mediciation intended to treat anxiety. The assessment will assess all relevant inclusion/exclusion criteria. This measure requires 15-30 minutes to complete. Subjects entering the treatment study will undergo venipuncture, though this is an optional procedure for subjects who are needle phobic. Finally, we may acquire salivary samples during the physical examination and at various points throughout the protocol during neuropsychological tasks and during fMRI or M/EEG imaging. These specimens will be used to assay various hormones.

7.5.1.3. *Core Neuropsychological and Symptom-based assessment:* Subjects will be assessed with a set of standard paper-and-pencil neuropsychological measures. For all participants, questionnaire research data will be collected and stored using the NIH Clinical Trials Survey System (CTSS) and the Clinical Trials Database (CTDB). Coded questionnaire data will also be transferred and stored on the Neuroinformatics Database. This allows parents and children to log in to a secure, password-protected website and directly enter responses to questionnaires and checklists, or enter their responses while at NIH using a wireless-device interface to access the NIH-intranet secure CTDB. With the exception of the WASI and CELF, when possible, these will be repeated after treatment. This will allow better characterization of the sample and facilitate comparisons between performance on standard, population-normed neuropsychological tests and

experimental paradigms. Moreover, measures of acute, anxiety-related clinical status, including the Pediatric Anxiety Rating Scale (PARS) and the Clinical Global Improvement (CGI) scale, will be completed both before and after treatment as well as mid-treatment. In addition, for patients with depression, measures of depression will be completed both before and after treatment as well as mid-treatment. Self-reported measures of anxiety (SCARED) and depression (CDI/MFQ) also will be completed both before and after treatment as well as mid-treatment. For all other measures, involved in characterization of other patient characteristics beyond acute anxiety that is expected to respond to treatment, these will be completed when most convenient for the families. The questionnaire may be done in person or online.

Depending on participant age and tasks being completed, standardized tests may include any combination of the following: i) the WASI screening intelligence test; ii) the Clinical Evaluation of Language Fundamentals screener (CELF-3 Screener); iii) the Benton Facial Recognition Test, iv) the DANVA-2 test of facial affect recognition; v) the Mayer, Salovey, Caruso Emotional Intelligence Test (MSCEIT), vi) State Trait Anxiety Inventory (STAI); vii) Beck Depression Inventory (BDI); viii) Attributional Style Questionnaire (ASQ); ix) Marlowe-Crowne Social Desirability Scale-Short Form (SDS); x) Children's Depression Inventory (CDI); xi) Screen for Child Anxiety Related Emotional Disorders (SCARED); xii) Multidimensional Anxiety Scale for Children (MASC); xiii) State Trait Anxiety Inventory for Children (STAI-C); xiv) Children's Attributional Style Questionnaire (CASQ); xv) Pleasure Scale for Children (PSC); xvi) Children's Social Desirability Scale (CSDS); xvii) Gordon Test of Visual Imagery Control; xviii) Snaith-Hamilton Pleasure Scale (SHAPS); xix) Children's Communication Checklist (CCC); xx) Autism Screening Questionnaire (ASQ); Social Skills Questionnaire (SSQ); Spielberger State/Trait-revised (CDRS-R), Marlowe-Crowne Social Desirability Scale (SDS), Abbreviated 10-item Connors Teacher Questionnaire (ATQ), Conners' Parent Rating Scale – Revised; the Young Mania Rating Scale; Bis/Bas Scale, the Emotional Intensity Scale (EIS), the Berkeley Expressivity Questionnaire (BEQ), the Sensitivity to punishment and sensitivity to reward questionnaire (SPSR), the Parenting Questionnaire (Parent and Child versions), the Rigidity Questionnaire (Parent and Child Versions), the Spence Children's Anxiety Scale – Parent Report (SCAS-P) and – Child Report (SCAS-C), the Intolerance of Uncertainty (IUS), Intolerance of Uncertainty for Children (IUSC), the Penn State Worry Questionnaire (PSWQ), and the Penn State Worry Questionnaire – Child (PSWQ-C), the Childhood Anxiety Sensitivity Index (CASI), the Anxiety Sensitivity Index (ASI), the Family Accommodation Scale Anxiety (FASA; Child- and Parent-Report), the Spider Phobia Questionnaire (SPQ-C), the parent-report and child-report versions of the Difficulties in Emotion Regulation Scale (DERS), the State-Trait Anger Expression Inventory-2 (STAIXI-2 C/A), the Attention General Questionnaire (Parent and Child), the Sensation Seeking Scale from the Zuckerman-Kuhlman-Aluja Personality Questionnaire (ZKA-PQ), the Leibowitz Social Anxiety Scale (LSAS), Social Anxiety Scale (SAS), the Mood and Feelings Questionnaire (MFQ; Parent-, Child-, and Adult-Report), the Self-Efficacy Questionnaire for Children (SEQ-C), the Affective Reactivity Index (ARI; Parent-, Child-, and Clinician-Report; 1 week and 6 month)[145], the Single-Item Math Anxiety Scale, the Self-Math Overlap Measure, The Creativity Anxiety Measure, the Beck Anxiety Inventory (BAI), the Behavioral Inhibition Questionnaire (BIQ Child and Parent), Intolerance of Uncertainty Scale (IUSC), Positive and Negative Affect Scale (PANAS-C), Penn State Worry Questionnaire (PSWQ-C), Temperament in Middle Childhood Questionnaire (TMCQ), the Fear of Pain Questionnaire (child report), Fear of Pain Questionnaire (adult report), the Overcontrol in Youth Checklist, the Loneliness and Aloneness Scale for Children and Adolescents, the UCLA Loneliness Scale, the Media Use Questionnaire (child and parent report), the Problem Video Game Playing Scale (child and parent report), the Vanderbilt Life Events Checklist (child report), the Emotional Eating Scale – Child (EES-C), the Loss of Control Eating Disorder (LOC-ED) Screening Questionnaire (child report), the Thoughts Questionnaire (child report), the Public Speaking Anxiety Questionnaire (child report), the Child Avoidance Measure (child and parent report), the Emotional Avoidance Strategy Index for Adolescents (child report), the National Stressful Events Survey Acute Stress Disorder Short Scale (child report), the Positive Risk

Taking Scale (child report), the Brief Fear of Negative Evaluation II and Straightforward Items (child report), Fear of Negative Evaluation (FNE), Fear Survey for Children – Revised (FSSC-R), Development and Well-Being Assessment (DAWBA), Strengths and Difficulties Questionnaire (SDQ), the Adolescent Social Cognitions Questionnaire, Adolescent Social Behavior Questionnaire, Performance Anxiety Questionnaire, the Spatial Anxiety Measure, Post-Task Single Item Anxiety Measures, Tempo-test Atomatisieren (TTA), Rapid Single-Word Reading Measure, Mental Rotation Task (MRT), the Alternative Uses Task (AUT), the Self-Reported Grades Form (child and parent), the Authorized Deception Survey, the Coronavirus Impact Scale (CIS), the CoRonavIruS health Impact Survey (CRISIS), the Repetitive Behavior Scale (RBS-R), the Detail and Flexibility Questionnaire (DFlex), the Attention Bias Questionnaire (ABQ), the Patient Health Questionnaire-9 (PHQ-9), the Youth Exposure Efficacy Scale for Anxiety (YES), the Eating Disorder Examination Questionnaire (EDE-Q) and the Eating Disorder Examination Questionnaire adolescent version (EDE-Q-A), the Emotional Eating Scale for children and adolescents (EES-C), the Child Behavior Checklist (CBCL), and the Five Facet Mindfulness Questionnaire-Short Form (FFMQ-SF),

Parents of children enrolled in the protocol may complete any combination of the following: Alabama Parenting Questionnaire (APQ), Assessment of 6 major dimensions of personality (HEXACO), Symptom Checklist 90 Revised (SCL-90 R), Cognitive Error Questionnaire (CEQ), Cognitive Emotion Regulation Questionnaire (CERQ), Delay Discounting Test (DDT), Detail and Flexibility Questionnaire (DFlex), Difficulties in Emotion Regulation Scale (DERS), Frustration Discomfort Scale (FDS), Frustrative Non-Reward Scale (FRS), Interpersonal Reactivity Index (IRI), Parenting Scale (PS), Parental Sense of Competence Scale (PSCS), Parenting Stress Scale (PaSS), Perceived Stress Scale (PeSS), Reward Responsiveness Scale (RRS), Social Information Processing-Attribution and Emotional Response Questionnaire (SIP-AEQ), State Impulsivity Scale (SIS), Symptom-Checklist-90 Revised (SCL90-R)

A set of measures is acquired that relates directly to levels of stress exposure and sensitivity. These measures quantify common stressors previously shown to predict mood and anxiety symptoms. Specifically, we will assess exposure to stress using four scales: The Life Experience Survey (LES), the Family Risk Factor Checklist (FRFC), the Assessment of Perceived Events Schedule (APES), a self-report measure for distinguishing major and daily life events allowing adolescents to rate the positive or negative impact of each event, the Traumatic Experience Schedule Interview (TESI), and the Stressful Life Events Scale (SLES-C child and SLES-P parent). The first three measures represent checklists that have been used frequently in previous research on stress in children and adolescents. The TESI supplements questions on trauma that appear in the KSADS. These measures assess similar constructs as those that are quantified by the pandemic-related measures, the CIS and the CRISIS. However, whereas the main stress measures included in the study quantify reactions to many common forms of stress, the CIS and CRISIS assess stress specifically related to the COVID-19 pandemic.

We will assess aspects of attentional control using these two scales: Attentional Control Scale for Children (ACS-C) and the Early Adolescent Temperament Questionnaire-Revised Long Form (EATQ-R). We also would like to assess empathy with Bryant's Index of empathy in children and adolescents and the Griffith Empathy Measure, which asks a parent to rate their child's empathy. We also would like to add five questionnaires that will assess three factors related to peer acceptance and rejection. These factors include self esteem, as measured by the Rosenberg Self-Esteem Scale (RSS), social status, as measured by the MacArthur Social Status Scales (youth version), current and past exposure to peer victimization, as measured by the Kids In My Class Survey—Current and Kids In My Class Survey—Past, and bullying, as measured by the Olweus Bullying Questionnaire. The Kids In My Class Survey has been adapted to reflect increased communication through the computer and mobile devices, and thus includes questions about cyber-bullying. A second version of the Kids In My Class Survey has been added, allowing us to assess both current and past

experiences of bullying. *The Dating Anxiety Scale* (DAS)[139] addresses anxiety in both generic hetero-social contexts and specific dating contexts. All questions that are specifically related to dating will have the optional response of 'does not apply' for participants who are too young to have had dating experience. *The network of relationships inventory* (NRI)[140] asks several questions about the interpersonal characteristics of specific social dyads. Participants will fill out one that relates to a parent, one that relates to a friend, and one that relates to a boyfriend or girlfriend if they endorse having one. Finally the *Passionate Love Scale* (PLS)[141] is a brief 15 item questionnaire about the emotional intensity of romantic love. If participants don't report ever having been in love (first question) they will be instructed to leave the remainder of the questions blank. We also will administer the Diagnostic Symptom Questionnaire (DSQ) to subjects throughout the breathing test. Tests of memory will be augmented by a virtual reality spatial memory test[162]. In this test, participants are guided through a virtual environment and subsequently probed on memory of the path and landmarks throughout. Moreover, for some subjects, a test of sequential memory from the NIMH toolbox will be administered, whereby subjects will be required to remember the sequence of pictures[163]. Subjects may also be asked to complete a paired association task, whereby they will be presented with several pairs of images and then tested on the pairs[164]. For some subjects, a version of the sequential memory task and paired association task will be completed in the home. These subjects will view the task on a computer or tablet, and the subjects will complete a version of the task suitable for delivery in the home.Similarly, for some subjects, memory will be conducted while eye movement scanning patterns are assessed. This will occur at the time of other eye-movement paradigms. These measures require approximately one to two hours, in total, to complete.

Research suggests that ecological momentary assessments (EMA) provide unique information about anxiety symptoms. Some data suggest that, compared to in-clinic assessments, the EMA-based assessment is more sensitive to pathophysiological correlates of adolescent mental disorders[131], [132], [135]. Therefore, NIH will either provide subjects with a mobile smartphone device that is enabled to only record subject responses or subjects will complete assessments on their own smartphone device. The decision to use a NIH-provided smartphone or their own device will be based on the subject's preference. Assessments are accessed via a link to the questionnaire sent by text and responses are stored on a secure server. Responses will only be queried between 7am and 10pm on weekdays and on weekends, during times that have been selected for when participants are not in school. Subjects will be asked a set of 15 questions relating to recent experiences and emotions. The content for these 15 questions will be drawn from a pool of IRB approved questionnaires, listed in the preceding paragraph. No "actionable responses" will be queried. In total, it should take approximately one to two minutes for subjects to complete answers to these 15 questions. Two sets of EMA studies will be performed. In addition, questions will be added about memory for experiences during NIH visits. This should take one additional minute.

> In one phase of EMA research, data will be collected during up to five, one-week increments over a 2-year period, and subjects will be queried no more than four times on any given day. These assessments will occur for subjects who are currently undergoing or have completed treatment as part of attention-bias modification therapy (ABMT) and for healthy subjects. For those five weeks, subjects' EMA responses will be reviewed by an NIMH clinician at the end of each week, when subjects return the phone to the investigators. Subjects will be told to complete these questionnaires privately, when they are alone. Data for ecological momentary assessments (EMA) will be collected using secure technology, with procedures approved by the NIMH ISSO. This will involve direct collection of the answers on a secure server, and no data will be saved on the phones. This methodology sends text messages to participants to complete prompts. When participants opt to use their own phones, no data will be stored on the phone and all surveys will be accessed via text message. The technology for gathering the data will rely on mPath or RATE-IT. Both mPath and

RATE-IT have received approval from the NIMH ISSO. mPath is an application developed by the Katholieke Universiteit (KU) Leuven in Belgium who host the EMA data collected. Katholieke Universiteit (KU) Leuven in Belgium will not access to PII with the newly designed data system. Copies of the data will be stored at NIH on our secure servers and uploaded periodically (current schedule hourly). Specifically, all data is collected through the mPath application and stored in a mysql database. Data is initially stored on KU Leuven mysql server and periodically (hourly) uploaded to our server hosted on our GCP environment. Data on our mysql server is designated as the primary data and is subject to all federal and NIH data policies. Access to the database server is restricted to SIO staff and the firewall is configured only to allow connections from m-Path servers. RATE-IT is a mobile application that resides on the Amazon EC2 cloud service and is FedRAMP certified for government use. Research data is encrypted in transit using SSH/SSL certificates and it is encrypted in storage using the inherent Amazon encryption service. Text messages sent to participants to complete prompts will not contain any sensitive Personally Identifiable Information (PII) or Personal Health Information (PHI).

7.5.1.4. *Fear Conditioning & Avoidance Testing* (optional)***:***

7.5.1.4.a.*Fear Conditioning:* When confronted with fearful or unpleasant events, humans can develop fear of specific cues associated with these events through a process called classical conditioning. This aspect of the study examines fear responding in children and adults using a classical conditioning paradigm. The procedure is conducted in one of two settings, either in the MRI scanner or Dr. Grillon's laboratory. For studies in Dr. Grillon's laboratory and the MRI scanner, subjects undergo continuous recording of physiology (e.g., heart rate, skin conductance, and orbital EMG [eye blink startle]). We use three procedures to study fear conditioning. One of these relies on a loud scream as an unconditioned stimulus (UCS). The second uses one of various loud noises heard over earphones and aversive air-puffs, as a UCS. The third uses a thermode (http://www.medoc-web.com/products/pathway) to administer heat to the upper or lower extremity. The plan is to conduct one of these procedures in the psychophysiological laboratory and the other in the fMRI suite. However, depending on scanner availability, some subjects may receive the procedures only in the physiology laboratory or only in the MRI scanner. Moreover, other subjects may receive all three of the procedures in the MRI scanner. Procedures for the MRI scanner are similar to those in the laboratory, as described below.

Psychophysiological data, which will be collected in both the laboratory and MRI scanner, are measured, collected, and analyzed using PSU units from Contact Precision Instruments (London, U. K.) and PSYLAB software. Heart rate is recorded using two electrodes containing a standard neutral skin conductance paste. Skin conductance is recorded from the participant's non-dominant hand using two standard electrodes and attached to the ring and middle fingers. Orbital EMG is measured using fear potentiated startle in the psychophysiology laboratory and in the MRI scanner using signal intensity during passive monitoring in the facial region from two electrodes. Orbital EMG data in the psychophysiology laboratory will be analyzed by studying changes in average level of fear-potentate startle as a function of stimuli presented, whereas changes in average amplitude for specific stimuli will be analyzed in the MRI scanner. Also in the laboratory, since corrugator response is so subtle, a webcam will be installed and used to verify whether there are inherent differences in the EMG responses between trials where participants consciously look away or shut their eyes, which can elicit high corrugator activity, over the unconscious activity to the stimuli. This will be especially important to determine the validity of the trial responses for the EMG data. EMG data will be used as a secondary outcome in the study and serve as another unit of analysis for studying stimuli processing.

The scream-based conditioning procedure relies on five stages. A four-minute baseline is acquired followed by a habituation, preacquisition, and acquisition phase. During the habituation phase, subjects are presented with several air puffs (approximately 10 psi) to their forehead to acclimate the subject to this

assessment method. During the preacquisition phase, subjects are presented with 2 neutral stimuli (i.e., pictures of 2 adult female faces). They view several pictures each of the two different female faces. Following habituation, participants view these same two female faces, but one face is paired with a moderately aversive stimulus (i.e., a loud 95 dB scream) while the other is not paired with an aversive cue. All participants complete the final extinction phase either on the same day or on a different day. For the extinction procedures, presentations of each female face are given without a UCS. Moreover, this task will be conducted on at least two occasions, one of which will involve initial conditioning, followed by extinction, and a second of which will involve exposure to a few UCS presentations, followed by morphed presentation of CS+ and CS- stimuli. In addition, the extinction recall session, where morphed presentations without any aversive stimulus, may be repeated in separate sessions either in the psychophysiology laboratory or in the scanner. Of note, these faces may appear within the context of a virtual reality environment, using a platform adapted from the Morris Water Maze task described on page 18 to assess effects on navigation. Moreover, the CS+ and CS- stimuli also may appear as part of a button-choice and eye-movement task, using procedures from the dot-probe (see page 24) or behavioral avoidance (see page 17) tasks. Finally, the other two conditioning paradigms also may be used in this same fashion, within the context of a navigation, button-choice, or eye-movement tasks.

All procedures for conditioning using a scream UCS will be used in a second task with two changes. First, the UCS will appear at the lowest decibel sound volume possible, up to 95 db, similar to sounds that have been used in recently published conditioning studies.[71] This sound may or may not also be paired with the same aversive air-puff used in the emotion-regulation task described on page 18 and in prior published work from our group.[72, 73] One of a few sounds will be selected based on prior work. These may be either variants of white noise or an alarm with a 1000-Hz tone; the air-puff is delivered to the throat. The sounds might also be paired with previously approved scary pictures of animals from the IAPS dataset. Second, the CS+ and CS- are shapes presented in various combinations. They may be circles of either small or large diameter, as also used in a recently published conditioning study.[74] Alternatively, they may be other combinations of shapes, such as colored squares or triangles. As noted above, these and other conditioning procedures may be presented either in the psychophysiology suite or the MRI scanner. Moreover, after conditioning and extinction, extinction recall sessions also may be scheduled in or out of the scanner. Whereas the extinction-recall session for the fear-based tasks relies on morphs varying along facial-identity dimensions, the particular variant used for the aversive-sound task relies on morphs that vary in some physical parameter related to the CS. For example, if the CS+ and CS- are circles, than the morphs would vary in size between that of the CS+ and CS-. These procedures also follow those in published work. For some subjects, a version of the conditioning task will be completed in the home. These subjects will view the task on a computer while wearing headphones, and the subjects will complete a version of the task suitable for delivery in the home. Subjects will wear mobile technology at this time to record physiology.

The third conditioning task uses a thermode to deliver heat pain as the UCS. These procedures are based on an extensive prior literature performed by our collaborator, Dr. Lauren Atlas[116]. Dr. Atlas and her colleagues personally have used these procedures in hundreds of adult subjects without any notable adverse events; her colleagues also have used these same procedures in children and adolescents without any notable adverse events. These experiences from Dr. Atlas and her colleagues are consistent with reports in the published literature using these methods in many hundreds of children in the same ages to be studied in the current protocol[117, 118, 119, 122, 123, 124]. With this task, eye-blink startle will be probed using a standard startle stimulus used in prior research with children[126, 127]. This stimulus is a 40-50-msec duration 100-106 dB burst of white noise presented binaurally through headphones.

The successful application of these procedures across large samples and broad age range relates to two factors: the precise control of stimulus temperature with the apparatus used in these studies and the tailoring of stimulus temperature to subjects' individually-determined pain threshold. The current

protocol employs both features. The device used by Dr. Atlas, which will be used in the current study, is accurate to within 0.1 degree Celsius and includes an automatic safety shutoff device, built into the stimulator. This prevents exposure to heat that is capable of damaging the skin. Moreover, since our studies include children, a vulnerable population, we will rely on a less extreme UCS than used previously in work performed by Dr. Atlas and her colleagues. Specifically, in Dr. Atlas's prior research, a maximal temperature of 50 degrees Celsius for 10-seconds has been used, a level that is still not capable of producing tissue damage. In the current protocol, a maximal temperature of 48 degrees celcius will be used. Finally, as in Dr. Atlas's prior studies, we also will tailor UCS stimulus temperature to the subjects' individually-determined pain threshold. Due to the unique aspects of the UCS, these procedures for the heat-pain task differ from the procedures for the scream and bell task in two main ways. This concerns calibration of UCS level and details of conditioning using parametrically varying UCS levels.

In terms of UCS calibration, prior to the conditioning phase of the task, subjects will undergo the pain calibration task used in Dr. Atlas's prior work. This task requires subjects to rate pain induced by thermal stimulation using verbal ratings on a 10-point scale that varies from 0 (no sensation) to 10 (severe pain) during application of the thermode to eight sites on the left volar forearm in a series of 10-second trials. The calibration procedure will begin with a temperature of 34 degrees Celsius, and the temperature will not exceed 48 degrees Celsius. During conditioning, the aversive UCS will be calibrated to a rating of "8" for the subject. In terms of details of the conditioning procedure, the design for the heat-pain task generally conforms to the design for the bell and the scream task. However, procedures for the heat-pain task also will be adapted to conform to the procedures used previously[116], using two sets of stimuli. Namely, in one iteration of the task, CS+ and CS- will be tones rather than pictorial stimuli. In another iteration of the task, CS+ and CS- will be other pictures of abstract objects, faces, or scenes, drawn from the same pictures sets that will be used in other aspects of this protocol. Moreover, for the CS+, some trials will use a CS+ that predicts the UCS with a 100% reinforcement rate. Other trials will use CS+ cues that predict the UCS on some trials but a lower level of heat-pain on other trials. In another portion of the task, some cues will predict UCS onset with 100% certainty in a certain subset of trials, whereas these cues will show no relationship to UCS onset in another subset of trials in the task. Moreover, to increase the level of unpredictability in this part of the task, the heat pain also will be paired with other stimuli in the protocol to be used as a UCS. For example, a heat-pain UCS may occur at one point, and a sound UCS (scream, white noise) may occur at another; similarly, two UCSs may occur next to each other, such as a sound UCS immediately before a heat-pain UCS. The sound UCSs will be played at a volume level no higher than that approved in the protocol. This task has been used previously among youth, though prior uses of the higher uncertainty portion of the task in youth did not use the heat UCS[149]. Finally, subjects will rate pain levels after each UCS. Of note, as described below in the section on reward tasks, we also will use comparable procedures with similar CS+ and CS- with rewarding as opposed to aversive UCS.

7.5.1.4.a. Avoidance Testing **(optional)*:*** After conditioning, subjects also may undergo an avoidance task based on a previously used video-game task[125]. This task relies on a video game that is presented to subjects immediately after conditioning. However, while van Meurs et al. relied on shock UCS, the current protocol relies on a UCS used during one of the three conditioning tasks, where the UCS is less aversive than shock. In this video-game task, subjects engage in a role-playing game where they attempt to perform a task on each trial (e.g., harvest crops before chickens eat them). Each trial offers them a choice, between a relatively slow execution of the task resulting in a lower likelihood of winning the task but no risk for UCS exposure, and a more rapid execution of the task, where they have a much higher chance of winning but they risk UCS exposure. Avoidance testing may also be embedded in the context of other tasks, where aversive stimuli from these other tasks may also be used (e.g., loss of money; emotional photographs; cues from a social interaction task).

7.5.1.5. *CO_2 challenge task.* As noted above, the CO2 challenge task will be used to examine relationships among specific diagnosis, attention-based, fear conditioning, and physiologic procedures. The CO2 challenge procedure only will be completed once. Moreover, this procedure only will be used with children who are at least nine-years old, based on prior experience in this age group. All research participants will be invited to participate in a brief 7.5% CO_2 challenge task that has been used extensively in adults as well as several child studies.[104 105] This will generate physiology data that can be compared with physiology data acquired across other aspects of the current protocol, such as during conditioning. Most previous studies in youth relied on 5% as opposed to 7.5% CO2. However, work by our collaborator, Dr. Roberson-Nay, found that the reliability of the 7.5% CO2-inhalation procedure in adults was greater than found previously with the 5% procedure. As low reliability seriously hinders research, this led Dr. Roberson-Nay to begin using the 7.5% CO2-inhalation procedure with youth. As of December, 2013, Dr. Roberson-Nay had studied 110 youth in the age range that will be studied in the current protocol with the 7.5% procedure; she has noted excellent tolerability, with no greater rate of adverse events than seen previously with 5% CO2; the strong majority of her subjects return to complete a second procedure. Moreover, she finds similarly strong reliability in youth as she found previously in adults. Finally, the pattern of findings in youth for measures of anxiety with 7.5% CO2 replicate the pattern found previously with 5% CO2. This provides a strong rationale for using 7.5% CO2 in the current set of studies.

The task includes breathing air through a facemask in which the level of CO_2 is increased from ambient levels (less than 1%) to 7.5%. The air tanks will contain 7.5% CO_2 which will make it impossible for subjects to receive more than 7.5% CO_2 during the task. Although the inhalation of $CO_2$0-enriched air generally results in some level of physiologic arousal (e.g., increased breathing rate, increased heart rate) and can be anxiogenic, most children who undergo the task generally report very minimal levels of anxiety. This low rate of anxiety is documented when measured on a 0-8 point scale, with 8 being the highest. In the event a child experiences a panic attack or a panic-like response, the CO_2 challenge task will be terminated and the subject will be seen by a clinician. Dr. Pine will be notified.

During the CO_2 task, all participants will sit in a comfortable chair to minimize movement artifacts and maintain a consistent supine posture. Respiratory rate will be measured via a belt placed around the child's lower diaphragm (over the shirt) to measure diaphragmatic movement. Tidal volume will be assessed on a breath-by-breath basis through a transducer connected to the facemask. Heart rate and skin conductance will be monitored continuously throughout the challenge as well. Heart rate will be measured using two pre-gelled disposable electrodes placed on the participant's right wrist and left collarbone while two pre-gelled disposable electrodes will be placed on two of the participant's fingers to measure skin conductance. All physiologic data will be collected using the Biopac MP150 Data Acquisition System while all physiologic data will be analyzed with Biopac's AcqKnowledge software.

The CO_2 procedure requires that the participant wear a facemask throughout the experimental procedure. The facemask will be connected via gas impermeable tubing to a two-way stopcock valve, allowing the experimenter to manually switch from room-air to the CO_2 mixture. Connected to this valve will be a large multi-liter bag that serves as a reservoir for the 7.5% CO_2-enriched air. Subjects will breathe room air for 5 minutes followed by 8 minutes of 7.5% CO_2-enriched air and a 5-minute recovery period. Subjects will be unaware of ambient air versus CO_2 timing.

The entire procedure will be conducted in a quiet room located in the outpatient clinic (OP-4) of the Intramural Research Program at the NIMH Clinical Center in Bethesda, Maryland. A study experimenter will remain in the room with the child throughout the entire procedure. All participants will be instructed to signal if they feel uncomfortable or if they wish to terminate the CO_2 inhalation task for any reason.

In addition to physiological measurement, we will assess subjective anxiety throughout the task to monitor anxiety levels using a 0-8 point scale. We will obtain an anxiety rating every two minutes starting at baseline through the recovery period. The Diagnostic Symptom Questionnaire (DSQ) will be administered by the researcher to assess presence/absence as well as intensity of panic-relevant symptoms (e.g., dyspnea,

sweating, tachycardia). The DSQ will be re-administered at multiple time points throughout the experiment to track anxiety reactivity before, during, and after the CO_2 challenge task. Panic response also will be monitored by the study experimenter who will determine whether a participant has experienced a panic attack in response to the CO_2 inhalation task. As in prior studies, panic attacks require an increase in self-rated anxiety and increases of 1 point or more on at least 4 API symptoms. The main study hypothesis is that children and adolescents with anxiety disorder will show greater attention to the physiologic perturbation than healthy children and adolescents. This enhanced attention will be primarily reflected in responses on the DSQ, particularly in patients with separation anxiety disorder. However, secondary analyses will also examine physiological parameters and associations among response to CO2 inhalation and the other threat paradigms.

7.5.1.6. *Continuous Performance Task (CPT), Attention Filtering Tasks, and Attention Training Procedures* (optional): The CPT tasks represent standardized tasks involving trials of button pushes or eye movements and withholding of button pushes and eye movements. Specifically, we will use the CPT Trilogy tasks developed by Bilder and colleagues; the Stop and Change tasks implemented by our group in collaboration with Dr. Leibenluft; and the Ventral Attention Network task developed by our collaborators, Drs. Sylvester and Luby. In this task, participants view a central fixation point and then either press a button or make a saccade to a peripheral target; in some trials, only a fixation and target appear; in other trials, fixation is followed by targets amidst peripheral, distracting stimuli. Finally, a standard test of language pragmatics will be used. Of note these tasks are common to both this protocol and Dr. Nathan Fox's protocol 01-1115 and may be performed either at University of Maryland or NIH, and data will be shared between the two sites to prevent duplicate testing. For some subjects, a version of the Continuous Performance Task (CPT) will be completed in the home. These subjects will view the task on a computer, and the subjects will complete a version of the task suitable for delivery in the home.

7.5.1.7. *Cognitive-Emotion Interface Tasks* (optional):

7.5.1.7.1. General Rationale: The amygdala is involved in learning. In line with this, patients with amygdala lesions show impaired performance on three measures of instrumental learning used by Dr. Blair (e.g., the Tokens task, passive avoidance and the instrumental reward/punishment task). The suggestion is that the diminished amygdala signal reduces ability to associate particular tokens with reward/punishment. It is important to investigate instrumental learning in mood and anxiety disorders. One set of two tasks implemented by Dr. Blair enable a test this prediction. Through interactions with the prefrontal cortex, the amygdala is also thought to play a role in cognitive-emotion interface. In another set of two tasks, Dr. Blair has developed procedures for examining the impact of emotional stimuli on goal directed cortical processing. These tasks are specifically hypothesized to quantify the manner in which emotional stimuli alter frontally based goal-directed processes. One of these tasks involves a manipulation of the Number Stroop task, while the other examines the impact of emotional stimuli on an automatic motor response. Of note, these latter two tasks rely on IAPS stimuli. We will employ the set of IAPS stimuli currently approved by the IRB for use in this protocol in other tasks. Finally, a set of other tasks will examine the interface between cognitive and emotional processes, an interface mediated by the amygdala. Here, subjects' behavioral performance will be assessed in the context of emotional and unemotional task-related information.

7.5.1.7.2. Tasks:

7.5.1.7.2.1 *Passive avoidance* Each trial involves the participant deciding whether or not to respond (press the spacebar) when a given stimulus is being presented on the computer screen. Responding to four of the eight stimuli elicits reward (point gain), responding to the other four elicits punishment (point loss). In each block of trials all eight stimuli are presented to the participant. The experiment involves ten blocks of trials. In passive avoidance paradigms, instrumental learning is assessed by measuring rates of passive avoidance errors (i.e., responses to stimuli paired with negative reinforcement) and omission errors (i.e., failures to respond to stimuli paired with positive reinforcement).

7.5.1.7.2.2. *Probabilistic Response Reversal (PRR*): During the response reversal task, participants are asked to choose between two to three image stimuli presented on the screen on each trial. Stimuli will be paired with different probabilities of reward or loss, e.g. one stimuli may be rewarded 80% of the time that it is selected while another is rewarded 20% of the time. During a given block, these probabilities may switch, e.g. a stimulus previously rewarded 80% of the time is now rewarded only 20% of the time. These probabilities are all veridical and no deception is involved in the task. The task will take approximately 10-30 minutes to complete. Participants may be incentivized by receiving payment up to $25 of their winnings on the task. Accuracy and reaction time data will be collected to assess participant's ability to learn these incentive contingencies and then to learn and update after a change in contingencies. This data will be compared across the phases of the task and across patient groups. Additional computational modeling approaches will be used to estimate parameters about participants' behavior, like learning rate.

7.5.1.7.2.3. *Emotional Number Stroop:* In the Number Stroop task used, participants have to say which of two serially presented stimuli contain more numerals. There are three forms of Number Stroop trial: congruent, where numerosity agrees with magnitude (e.g., one stimulus has three 4s and the other five 6s); distance 1 incongruent, where there is a numerical distance of 1 between the numerosity and magnitude value (e.g., three 4s versus four 3s) and; distance 3 incongruent, where there is a numerical distance of 3 between the numerosity and magnitude values (e.g., three 6s versus six 3s). Both numerical stimuli are presented for 400ms. In between these stimuli, a visual image taken from the IAPS is presented for 400ms. These images are either of positive, negative or neutral emotional content. In healthy individuals, the presentation of an emotional image significantly impairs performance, particularly for distance 1 trials, relative to the presentation of neutral images.

7.5.1.7.2.4. *Emotional Interrupt*: This task assesses the impact of emotional processing on relatively habitual motor performance. Participants are engaged in a simple motor response task (left and right button presses to circle/ square stimuli). Before and after the presentation of the stimuli, the participant is presented with distracter stimuli that are either of positive, negative or neutral valence. The stimuli are presented serially: a distracter stimulus (200ms) followed by a response signal (150ms) followed by the same distracter (400ms). Participants respond more slowly to the target circle/ square stimuli if the bracketing distracter stimuli are of either of positive or negative valence than if they are of neutral valence.

7.5.1.7.2.5. *Behavioural avoidance task:* This task aims to assess the degree to which subjects are able to acquire associations between a neutral stimulus (colour card) and an emotional cue (angry face), and whether they subsequently learn to 'avoid' the neutral stimulus as a result of the emotionally aversive feedback. On the basis of existing findings in the literature, and preliminary results from a pilot study utilising this task, we hypothesise that subjects with anxiety conditions will show a bias towards a quicker acquisition of the association, and greater avoidant responses towards the neutral stimulus. The task is presented as a game to children, in which they are told to win as many points as possible by choosing between two different coloured cards.

7.5.1.7.2.6. *Social Interaction Task*: This task is 12 minutes long and combines face emotion ratings and viewing of social scenes with a simple motor task. While each scene is displayed, participants rate how well they performed on the motor task, based on depicted emotional expressions..

7.5.1.7.2.7. *Cambridge Neuropsychological Test Automated Battery (CANTAB):*

The CANTAB is well-standardized computerized battery comprised of subtests probing different aspects of cognition, including attention and memory. The battery has been used widely in studies of healthy and psychiatrically impaired children in the age range of the current protocol. Subjects respond to visually presented stimuli by pressing a touch sensitive screen. This battery probes aspects of memory and learning using a set of shapes and a touch screen.

7.5.1.7.2.8. *Conflict Adaptation Task*: Participants are presented with stimuli that combine fearful or happy faces with the word "FEAR" or "HAPPY" written across the face. Participants are asked to indicate the emotion of the face, while ignoring the distracting effect of the written word. Additional blocks present

other, non-emotional words as control trials. This task, which requires 15 minutes to complete, examines how subjects monitor and adapt to conflict from trial to trial. These cognitive adjustments, critical in daily life, are automatic. We are interested in testing whether these automatic processes differ in anxious individuals, and potentially contribute to the attention bias characteristic in anxiety disorders.

7.5.1.7.2.9. *Reward Tasks:* Impaired motivated behaviors lie at the root of maladaptive behavior in many psychiatric disorders. Adolescence is a key period during which many psychiatric disorders first emerge, and studies during this developmental stage may provide a unique window to address primary deficits associated with the disorders. We will examine the manner in which the various elemental emotional-cognitive processes are differentially affected in depression and anxiety, compared to healthy volunteers. The plan is to administer these reward paradigms in the clinic and in the fMRI suite; while all of the tasks listed below will be used in the clinic in some subset of subjects, up to six reward tasks will be used with fMRI. Procedures for the MRI scanner are similar to those in the clinic. Finally, subjects will be able to earn money while participating in these reward tasks. Subjects will complete anywhere from one to four reward tasks. Subjects will be allowed to earn no more than $150 total for participation in all reward tasks over a one month period. Earnings will be pro-rated based on the number of tasks completed.

7.5.1.7.2.9.1. Eye Tracking & Saccade Task:

Eye movements are measured with the model 504 eye tracking system developed by Applied Science Laboratories (ASL). The model 504 eye tracking system consists of a PC that serves as a user interface and a digital data recording device; a camera that captures eye movements; two video monitors, one for displaying an image of the eye, and one for displaying the scene that the subject views (with a set of cross hairs superimposed indicating the subject's point of gaze); a magnetic head tracker providing information about head position, that consists of a control unit, a transmitter module, and a small sensor attached to a baseball hat worn by the subject; and finally a model 5000 eye tracking control unit, which contains the processing board that integrates and coordinates all of the above-mentioned components. The eye tracking system is complemented by a second PC (the task PC), on which study subjects view stimuli and complete tasks. The camera measures pupil diameter and eye movements by capturing the eye's back-projection of a beam emitted by a ring of LEDs placed around the camera's lens.

Eye tracking is non-invasive, painless and poses minimal risk to the subject. During the task, subjects can move their heads within a small range, but are nevertheless prompted to move as little as possible. A chair specially designed to minimize movement and to support the subject's head is provided. Eye tracking will be used in as many tasks that are amenable to recording. Eye tracking will be used with the Saccade Task, described immediately below, in order to measure rapid voluntary eye movements towards and away from stimuli displayed on a computer screen.

In the Saccade Task, subjects view one of six possible stimuli. After a random time interval, the stimulus disappears and an asterisk appears either to the right or to the left of the central stimulus. Subjects are instructed to look as fast as possible to the asterisk if the previously displayed central stimulus was written in white ink, and to look to the mirror position of the asterisk if the previously displayed central stimulus was written in gray ink. They are informed that regardless of the color of the central stimulus, a plus sign signals that they will win $1 if they look to the correct location, a minus sign signals that they will lose $1.00 if they look to the incorrect location, and a circle signals that they will neither win nor lose money if they look to the correct location. The behavioral version of this task consists of approximately 3 runs of 4 minutes each. Each run consists of 52 trials. Feedback is provided after each trial. This task will be completed in conjunction with the eye movement camera. Subjects begin with zero dollars and end with an amount between 10 and 50 dollars. For some subjects, a behavioral version of the Eye Tracking & Saccade Task will be completed in the home. These subjects will view the task on a computer, and the subjects will complete a version of the task suitable for delivery in the home.

Affective versions of the saccade task will be used to examine the effect of affective stimuli and context on eye movement. These affective stimuli will consist in the presentation of visual cues (faces,

IAPS-type pictures, and complex social scenes, such as embarrassing moments or times when a child is excluded from a group, drawn from available data sources for child-appropriate stimuli), and affective context will consist of the combination of sounds and pictures (i.e., laugh with a happy face or scream with an angry face).

A subset of healthy subjects also will be invited to undergo training, designed to improve performance on the anti-saccade version of these tasks, using a version of the task where no money can be won by the subject. In the initial pilot phase, we will attempt to improve performance on the anti-saccade task by training on a version of the task that adapts the level of difficulty so that subjects' performance gradually improves. Other research[152, 153] successfully uses these training procedures to improve performance and brain function for tasks that engage inhibitory control processes, the same process engaged by the anti-saccade task. This initial pilot will involve two phases, both completed in healthy youth. The first phase will involve 10 to 15 healthy youth, trained to improve inhibitory control. This training phase will involve repeated administration of the antisaccade task where subjects perform increasingly difficult versions of the task, containing multiple distracting stimuli.

If the first phase of training is successful, a second phase will be implemented in another sample of 20-30 healthy youth, half randomly assigned to the active training regimen and half assigned to a condition that will involve repeated assessment with the antisaccade task. Subjects will complete eight 45-minute training sessions, scheduled based on subject convenience, to occur within one month.

7.5.1.7.2.9.2. Monetary Incentive Delay (MID) Task: The MID reward task consists of several trials of up to 10 seconds each, and takes approximately 5-10 minutes to complete, depending on the version employed. At the beginning of each trial, participants see one of three cues. Cues signal potentially rewarded response (1/3 of trials, cue 1), an unrewarded response requirement (1/3 of trials, cue 2), or no response requirement (1/3 of trials, cue 3). In some versions of the MID reward task, there is also a cue signaling potentially penalized responses. After the presentation of the cue, participants fixate on a cross-hair as they wait a variable interval, and finally respond to a white target square which appears for a variable length of time with a button press. Feedback that follows the target's disappearance notifies participants whether they won (or lost) money during that trial and indicates their cumulative total at that point. In the rewarded response trials, participants win monetary reward if they press a button during display of the subsequent target. In the non-rewarded response trials, participants do not win money for responding, but are still asked to rapidly press the button during display of the subsequent target. In the non-response trials, participants are asked to refrain from pressing the button during display of the subsequent target. In case of penalized trials, participants can lose monetary reward if they do not press the button fast enough during the display of the subsequent target. Target durations are adjusted such that each participant succeeds on approximately 66% of his or her responses, based on reaction times obtained during the practice session. Participants can make up to $25.00.

7.5.1.7.2.9.3. Signal Detection Task: In the signal-detection task, participants are told that the aim of the task is to win as much money as possible, and are then shown presentation of an asterisk for in the middle of the screen that served as a fixation point, then a mouthless cartoon face will be presented in the center of the screen. After a delay of 500 msec, either a short mouth (11.5 mm) or a long mouth (13 mm) will be presented for 100 msec. The face without the mouth remains on the screen until a key response is made. Participants are asked to identify which type of mouth is presented by pressing either the "z" key or the "/" key on the keyboard (counterbalanced across subjects). For each block, the long and short mouths are presented equally often in a pseudo randomized sequence, with the constraint that no more than three instances of the same stimulus were presented consecutively. Subjects can win up to a total of $10 in this task.

7.5.1.7.2.9.4. Loss Aversion Task: This paradigm provides a quantitative measure (lambda) of sensitivity to loss. This task requires subjects to decide between taking or dismissing a series of

monetary gambles. For example, subjects are asked whether they would take the gamble of 50% chance of a $5 gain vs. a 50% chance of a $10 loss, or prefer to pass on such gamble. This task is about 10 min long. In order to test appropriately the process of "loss aversion" we would like to provide an initial endowment of $10.00 that can be lost during the game. Subjects earn up to $40.

7.5.1.7.2.9.5. The Door Choice (DC) task: The Door Choice (DC) task is a computerized choice decision-making task involving choices where research participants must weigh the choice of probabilistic monetary outcomes against risk for aversive unconditioned stimuli (UCS). For the UCS, the same procedures from the conditioning task will be employed. Each choice is displayed as a door with two probabilities/magnitudes displayed on each side of the door. On one side, probability/magnitude of a monetary reward is depicted; on the other side, probability/magnitude of UCS is depicted. For the DC task, the thermode delivering heat pain stimulus used in conditioning tasks will be employed. A description of the thermal stimuli can be found in section in section 7.5.1.4 of this protocol. The temperature threshold for the UCS will be set using the same procedures from the conditioning tasks. However, subjects will be directly informed on the probability of UCS on each trial. Subjects perform versions of the task where they can win virtual points or money, in which subjects can only win or not win, or where they can lose points/money, in which subjects can only lose or not lose. In versions involving winning money, subjects are instructed that they will take home up to $50.00 of the money they win and that they should try to win as much money as possible or lose as little as possible. In each trial, subjects will see a door. This will reveal the possibility of winning or receiving the UCS if and when the door opens.

7.5.1.7.2.9.6. Behavioural Economic Task Set: The goal of this task set is to assess decision-making patterns in anxious and healthy adolescents and adults in standardized economic tasks. This is important to test the role of anxiety or adolescence in deleterious decisions. A set of 5 short screens will be given to participants. Screens will ask subjects to choose between (1) a sure gain of $5 vs. a 50% (coin toss) gain of $12; (2) a sure loss of $5 vs. a 50% (coin toss) loss of $12; (3) $4 now vs. $8 in a week; (4) a known probability of $4 gain vs. an unknown probability of $4 gain; (5) a known probability of $4 loss vs. an unknown probability of $4 loss. Each screen will have up to 4 choices with variable amounts and probabilities. Subjects can make up to $10 gain through this screen.

7.5.1.7.2.9.7. The Dual-Attention Working Memory (DAWM) Saccade Task: The goal of this task is to test the question of dissociation in the function of the ventral and dorsal attention pathways as a function of age (e.g., adolescents vs. adults) and anxiety. The stimulus-driven, ventral pathway is expected to be more dominant in adolescents and in anxiety than the goal-driven, reflective pathway. This theory will be tested in conditions of various cognitive loads, using a working memory task with different levels of difficulty. The stimuli include a fixation cross, central cue, and peripheral target for the eye movements, and for the memory task a series of simple shapes. For each trial, participants are presented first a memory cue (e.g., 1, 2 or 3 shapes), then is asked to perform a prosaccade (or an antisaccade), and finally is asked to remember which shape they saw. The task lasts about 23 minutes.

7.5.1.7.2.9.8. Reward Tasks: We will use a specially built MR-compatible, pneumatic-pressurized tastant-delivery system which has been previously approved for use by NIMH (PI: Alex Martin) to deliver tastants that are sweet, neutral, or bitter. The sweet tastant will be either apple juice or distilled water sweetened with sucrose. The neutral solution closely matches the properties of human saliva. Solutions will be used that have been employed at NIMH in ongoing research (sucrose: 0.4 M, 0.60 M, 0.8 M, and 1.0 M[144]; quinine sulfate: 0.125 mM, 0.3 mM, 0.4 mM, 0.5 mM[142,143]). Each solution first will be administered three times, in random orders. Subjects will rate the tastes based on pleasantness, unpleasantness, intensity, and sweetness. The averages of these three ratings will be used to find appropriate intensity and pleasantness. The neutral solution will also be used as a "wash" during the scan. Next, tastants will be delivered to the subjects' mouths through the tastant delivery system from syringes through latex-free hospital-grade plastic tubing into a gustatory manifold, with 0.4-0.8 ml of liquid.

In addition, three sets of reward-based tasks will be delivered to examine how people explore novel reward options based on work by Averbeck and colleagues[136, 137, 138]. The first is a three choice task where participants will select among three images that are paired with a probability of obtaining reward. Novel images will be introduced throughout the task. The second is the 'Horizons task' where participants will decide between two options that yield different probabilities of reward. The third is a 'sequential sampling task' where participants will see sequential offers for items at varying prices and will be rewarded for selecting the item with the best value.

7.5.1.7.2.10. *Emotion Regulation Tasks*: We will augment our IAPS regulation procedure by measuring the startle reflex, utilizing methods already approved for use in Dr. Christian Grillon's protocol 01-M-0185. We will assess eye-blink magnitude, skin conductance rate, heart rate, and respiratory rate non-invasively during the viewing of the Pediatric International Affective Picture System (IAPS) images that the IRB has approved for use with adolescents as part of this protocol. We also plan to measure the startle reflex in the context of threat employing the procedure approved for use by Dr. Christian Grillon (01-M-0185). However, instead of administering an electrical shock, as the aversive stimulus, we will direct a burst of air at an intensity of 60psi at the larynx. This air puff procedure previously has been approved by the IRB, and we found that it is tolerated well by adolescents.

At the beginning of the study subjects will be told that those participants, who most successfully regulate their emotion (through startle modification greater than 20%), will receive 30 dollars in addition to the standard reimbursement received by participants in this protocol.

7.5.1.7.2.11. *Spatial Navigation Task[162]:* The hippocampus is known to be a crucial region involved in spatial memory. The spatial navigation task has two versions, which will be counterbalanced. Both versions contain the same stimuli and instruct subjects to view a 69-second, first-person perspective video where they are walking through a forest-like environment until they reach a rocket ship. In the first version, however, the task instructions provided beforehand will describe the environment as neutral and non-threatening. While in the second version, subjects will be provided a narrative about the environment as hostile and threatening. Along the route they pass several distinct landmarks (e.g., a boat, car, etc) and intersections. Prior to the start of the task subjects are instructed to pay attention and told that they will be tested on their memory of the route taken in the video. Immediately following the video, subjects are tested on several aspects of spatial memory related to the video (e.g., landmark location, location-allocentric, path route, etc). In total the task will not exceed 20 minutes. For some subjects, a version of the spatial navigation task will be completed in the home. These subjects will view the task on a computer, and the subjects will complete a version of the task suitable for delivery in the home.

7.5.1.7.2.12. *Attention Blink:* This task provides a measure of how emotional stimuli alter perception. Subjects see a series of stimuli presented rapidly (every 100 ms) comprising 12 scrambled faces interspersed with 2 faces having a neutral or negative (fearful or angry) emotion. At the end of the series (labeled a "trial"), subjects are asked questions about what they saw (e.g., did you see a face? Was it a boy? Did you see a second face?). Subjects perform 20 trials, each with face stimuli that are both neutral, first neutral and second emotional, or first emotional and second neutral. When the 2nd face stimulus is presented at an interval less than 500ms from the first face presentation, this 2nd face stimulus is not perceived. This time interval is shorter when the 2nd face is an angry face, suggesting that the emotional salience of the 2nd stimulus affects attentional processes. We expect this task to be sensitive to anxiety disorders and provide another way to assay neural correlates of anxiety. This task lasts about 12 min.

7.5.1.7.2.13. *Yale Interactive Kinect Environment Software (YIKES) Task:* This task also assessing the effect of threats on attention in the context of an interactive videogame[146]. This videogame projects the subjects image into the game, and subjects move their image through a video interface with the computer, as described by Leibowitz and colleagues. Briefly summarized, subjects stand in front of a TV, and their image is projected on the TV through a video monitoring device. To succeed in the game, subject must move their

body to the left and right by shuffling, so that their image on the TV also moves to the left and the right. This allows their image to avoid balls that are falling from the top of the image on the TV. Images of dangerous animals, faces depicting photographs, or other stimuli used in other tasks also appear on the TV. Attention is assessed by examining the effects on subjects' movements that occur when these objects appear. During some runs of the task, we will concurrently collect eye-tracking data to measure attention to the various stimuli presented during the task. For this purpose, we will use Tobii Pro Glasses 2, which allow for the assessment of eye movements while the participant is in motion. This technology utilizes a pair of eye-tracking glasses attached to a light-weight, portable recording console and a separate laptop to control the glasses' operations. We will also use Empatica E4 wristbands to measure blood volume pulse, motion-based activity, electrodermal activity, and peripheral skin temperature throughout the task. This portable technology allows us to track physiological changes that occur during the task. We expect this task to be particularly engaging for children, as reported by our collaborator, Dr. Leibowitz, who developed the task. Moreover, we expect that children and adolescents with increasingly high levels of anxiety symptoms will show increasingly large effects on movement from these images.

7.5.1.7.2.14. *Executive-Emotion Tasks*: The current protocol collects data using some executive function tasks both in neutral conditions, as described above, as well as in frightening circumstances. This will quantify the effects of fear on executive function. Six sets of executive function tasks will be delivered. This includes the above-noted working memory (p. 16), Stroop (p. 15), saccade (p. 17), and attention filtering (CPT p. 14) tasks as well as a choice-guessing task developed by Averbeck and colleagues, where subjects will have to choose objects either to obtain point rewards or avoid fear-inducing conditions, and a modified Effort-Expenditure for Rewards Task (EefRT). The modified Effort-Expenditure for Rewards Task (EefRT) is considered and objective measure of reward motivation. In this task participants are presented with a series of repeated trials in which they are able to choose between performing tasks of varying difficulty in order to earn rewards, such as money or points. A modified version of the task will be used, where participants either have to make button presses or grip a hand dynamometer (a device that measures the force of a participant's hand squeeze) to acquire a reward. If participants successfully complete a trial then they are eligible to receive the reward allotted for that trial, which will vary between an equivalent monetary value of $0.00 and $5.The task may contain blocks of effort choice, where participants choose between an easy task (press a button a few times or squeeze a little) and a hard task (press a button many times or squeeze a lot), or a choice between no action and an action (i.e. 'effort'). Each trial will display the options and indicate what may be won. The initial information phase will be followed by a 'ready' screen where participants may complete the action, either finger press or dynamometer squeeze. Subjects are informed if they successfully complete the action and a feedback screen then indicates the outcome of that trial (reward feedback). Total possible winnings will not exceed a monetary equivalent value of $25, in addition to $20 for simply performing the task. The total time for the task is approximately 35 minutes (15 minutes of training and 20 minutes of task performance).

All six tasks will be administered in two conditions: a neutral condition and a fear-inducing condition. During the neutral and fear conditions, subjects will be presented with several air puffs (approximately 10 psi) to the forehead (p.14) or a low-level auditory probe. The fear condition will also consist of exposure to either a visual-auditory stimulus (a bell sound, or scream paired or not with a fearful face, p. 13-14), or a somatosensory stimulus (an air-puff delivered to the neck). Physiological measures of heart rate, skin conductance, and eye blink reflex will be collected throughout the study (as in p.14).

7.5.1.7.2.15. Countdown Task: This short, 15-minute task will be administered to support components of the protocol currently being performed across the NIMH, University of Wisconsin, and Vanderbilt University. This task indexes physiological responses, specifically skin conductance and eye blink reflex, during certain and uncertain anticipation. In this task, participants will see two-minute blocks of

negative and neutral IAPS images separated by an image of a clock that either counts down to the presentation of the image stimulus (certain timing) or clocks in a random order (uncertain timing).

7.5.1.7.2.16. Affective Posner Task: The current protocol examines the interface between changes in emotion and attention. The protocol uses a number of tasks where prior data document perturbations in children with anxiety. However, in other research, perturbations also occur in children with disruptive behavior disorders. The Affective Posner Task represents one area of research where children with behavior disorders exhibit perturbed attention. The task requires participants to respond to targets that generate varying levels of reward and punishment, producing frustration i.e. failure to earn reward. The task is organized into three blocks consisting of a series of timed-response trials. At the end of each trial, children receive feedback as to the accuracy of their responses and, on trials during the second and third blocks, they win or lose money on each trial. On all trials, a fixation screen is inserted between the target/response and the feedback slide. During the first block (70 trials) the child is told to respond as quickly as possible to the stimuli and that there will be no reward or punishment, but they receive feedback on their accuracy. During the second block (110 trials), participants are introduced to the monetary contingency and win or lose $0.50 when they make a correct or incorrect response, respectively. The third block (condition 2; 240 trials), is the frustration condition, during which feedback is rigged so that 60% of correct trials are followed by negative feedback and a loss of $0.50 and 40% of correct responses are followed by positive feedback and a gain of $0.50. Trials in which participants make incorrect responses are followed by accurate feedback. Finally, fixation trials, during which participants focus on a fixation mark in the center of the screen, are included to facilitate comparisons between brain activity in win/lose trials with those in "baseline" states.

The task does involve deception in the frustration phase rigged trials. As with all our deception task procedures, the rationale for the deception is explained in detail to both subjects and parents once the last administration of the task has been completed, and we ensure that subjects and parents understand the reason for the deception. To minimize the potential for any negative effects related to the deception, a debriefing form is performed after task completion. Additionally, as is our practice, participants and their parents are given with the opportunity to exclude their data after the deception has been revealed. For participants completing the task thrice , debriefing will occur after the task is completed for the third time.

7.5.1.7.2.17. Vocal Affective Recognition Task: The current protocol examines the ability to recognize different emotional tones as expressed verbally by actors. Stimuli will consist of audio clips of female adolescents expressing brief phrases in different emotional styles. The phrases themselves were selected to be emotionally ambiguous in terms of content but can express very distinct meanings when delivered in different prosodic styles. For example on phrase used is "I can't believe you did that" can be used to express anger, joy, surprise, or fear when said in different ways and in different contexts. Previous tasks have demonstrated perturbations in emotion classification children with anxiety but they have relied primarily on visual depictions of emotion. This protocol will extend studies into the audio dimension.

7.5.1.7.2.18. *Dwell-Time Eyetracking Task*: Subjects will be studied using various techniques to quantify attention to faces, using various stimulus arrays, as assessed using eye-tracking and reaction time measures in various contexts. For relatively simple arrays of facial stimuli, this will include the dot-probe and saccade based tasks described in sections 7.5.2.1.2 and 7.5.1.7.2.9.1. In addition, for more complex stimulus arrays, subjects will be asked to complete a task where subjects will view matrices of faces with varying numbers. These matrices will contain mixtures of neutral faces and faces displaying various types of emotions. This paradigm will use faces drawn from the same data sets used for other face processing tasks in the protocol. We will quantify the duration during which subjects view negative as opposed to neutral or positive face emotion displays, to derive a measure of attention bias in context involving more complex arrays of faces than in either the dot-probe or saccade-based tasks.

7.5.1.7.2.19. *Social Flanker Task*: The protocol will use a traditional Eriksen Flanker Task with an added social element to examine how cognitive control and social arousal interact in anxious youth. A

previous study by Dr. Nathan Fox demonstrated that youth at risk of developing anxiety disorders have greater error related negativity signals (as measured by EEG) following response inhibition errors in the presence of a peer, compared to when they complete the task alone[147]. What remains unclear is if social arousal will influence behavioral and neural responses during the Flanker task in anxious youth. The social manipulation will involve deception, wherein subjects will be told that another subject is watching them complete the task. The subject will then participate in a verbal exchange of information (i.e., name, age, place of birth) with the other subject via intercom prior to starting the task[148]. However, the comments from the other subject will be pre-recorded audio clips that will be played by the researcher. Subjects will be debriefed following completion of all study tasks involving deception.

7.5.1.7.2.20. *Trier Social Stress Task*: Research participants will complete a version of the Trier Social Stress Test (TSST-C), where prior research finds the test to be well tolerated by children as young as eight[150]. The research participant will be told to tell a story, after a research assistant provides the beginning of the story, involving a visit to a scary neighbor's mysterious house against parents' wishes. The participant will speak for five minutes in front of NIH staff, who the participant had not previously met. The research participant will then be asked to conduct serial subtraction. The task will be videotaped; salivary cortisol will be collected; and peripheral autonomic physiology will be collected. The task may be administered in either an in-person or virtual format; in-person sessions will employ a judging panel in the same room as the participant while virtual sessions will employ a remote judging panel in which the participant views judges via video chat on a screen projected in front of them. Virtual TSST-C sessions will take place via Webex, a HIPAA-compliant teleconferencing platform. The audio of the Webex judging panel will be recorded for later analysis. Salivary cortisol and peripheral autonomic physiology will be collected in both virtual and in-person formats. Data, including salivary cortisol and video data, will be sent to Drs. Williams and Kalin at the University of Wisconsin. More information regarding this data sharing is noted in section 11.4: data sharing for secondary analyses, on page 51.

A week after the TSST, participants will be invited back and asked to watch back their own video recording of the TSST (Nikolic, de Vente, Colonnesi, & Bogels, 2016). We will also show them a video of a "peer" performing the task (in reality, this will be a confederate, not another participant). We will measure rumination, expectations, memory (what they remember from the TSST visit), and subjective anxiety using questionnaires, as these processes might play a role in the maintenance of anxiety (Clark & Wells, 1995; Leigh & Clark, 2018). We will ask them to evaluate their own performance and the performance of the peer (all participants will watch the same video of a confederate). We will collect more objective data by using eye-tracking and physiological measures (blushing, cortisol, heart rate, blood pressure, skin conductance response) and by filming the participants while they are watching their video (to automatically analyze facial expressions). We will also test if we can change how they perceive themselves (Goldin et al., 2014), by asking half of the participants to watch the video a second time while pretending it is somebody else. The other half of the participants will watch the video a second time without additional instructions.

7.5.1.7.2.21. *The Leiden Public Speaking Task:* This task will use the video from the Leiden Public Speaking Task, which displays eight students and one teacher in a simple classroom setting. Participants are asked to introduce themselves to the virtual class for one minute and to simply look at the audience for one minute. Participants know that it is not a real audience, and we will not tell them that they will be evaluated. Prior research has shown that this test elicits a moderate stress response in adolescents[154]. Eye-tracking data will be collected to measure attention to the faces of the audience members. For this purpose, we will use Tobii Pro Glasses 2, as previously discussed in Section 7.5.1.7.2.13. We will also use Empatica E4 wristbands to measure physiological changes throughout the task, as discussed in Section 7.5.1.7.2.13.

7.5.1.7.2.22. *The Motion Task*: In this task participants are presented with a series of face-emotion images, either an angry or a calm face. The face displays either direct (forward) or indirect (averted) eye-gaze. Over the course of each trial, the face image enlarges on the screen as if it were coming towards the participant. Paired with each face image is a sound that increases in volume as the face approaches. For

angry faces, the accompanying sound will be unpleasant (e.g white noise) for calm face images sounds will be neutral (e.g., a humming sound). Participants are told that they can "push the face away" on each trial by squeezing their controllers. Controllers are hand-held dynamometers, a device that measures the force of a participant's hand squeeze. When the participant squeezes the controllers, the face retreats and the sound softens. In addition to measuring hand grip force and latency to response, we will concurrently collect eye-tracking data to measure attention to the eye region of the face image. This is a behavioral task that will also be performed in the MRI scanner. There is no feature of deception. The total time for the task is approximately 20 minutes (5 minutes of training and 15 minutes of task performance).

7.5.1.7.2.23. Instrumental Learning Task: This task simulates a games and has multiple phases. In one phase, participants will view static colored images of objects (such as bells or balloons). The images will be different (e.g., colors), and will switch their location on the screen randomly. Participants will have two hand grip response devices in each hand and will grip one of the devices while the images are on the screen in order to select one image. After participants respond, the screen will acknowledge their selection by showing an image of a mallet overlaid on one image. Next, a feedback screen will indicate whether participants' choice is rewarded, such as "Win 50¢!" or "No Win." During this phase, participants learn to select the image that is followed more frequently by reward. In another phase, participants will view two static colored images on the screen. For some percentage of the participants' correct responses, the selection will be followed by a reward (such as "Win 50¢!"). For the other correct responses, feedback will be rigged, which will be followed by no reward (such as "No Win"). Incorrect responses will be followed by no reward (such as "No Win"). In total, the task will take approximately up to 50 minutes to complete. All images are original to the task and created graphically. One of the phases is designed to be frustrating. Children have tolerated other versions of this paradigm used in our group (Affective Posner task) without any adverse occurrences. As we do for other tasks (e.g., Affective Posner), children will be reminded at the beginning of the task, and at any point at which they report or appear too uncomfortable, we or they can stop the task anytime. Some participants may be asked to complete the task twice. Those who are enrolled in protocol 15-M-0182 and participating in Cognitive Behavioral Therapy (CBT) will be invited to complete the task once prior to CBT and once following CBT. The Frustration phase of the Instrumental Learning task is designed to be frustrating. Children have tolerated other versions of this paradigm used in our group (Affective Posner task) without any adverse occurrences. In any case, children will be reminded at the beginning of the task, and at any point at which they report or appear too uncomfortable, we or they can stop the task anytime. The task does involve deception in the frustration phase rigged trials. The rationale for the deception is explained in detail to both subjects and parents once the last administration of the task has been completed, and we ensure that subjects and parents understand the reason for the deception. To minimize the potential for any negative effects related to the deception, a debriefing form is performed after task completion. For patients completing the task twice, debriefing will occur after the task is completed for the second time, following CBT. Additionally, as is our practice, participants and their parents are given with the opportunity to exclude their data after the deception has been revealed. Participants can earn up to $25 based on their performance during the task.

7.5.1.7.2.24. *Profile Task:* In this task participants are presented with a series of neutral face-emotion images, facing the participant or portrayed in profile. Control stimuli are oval shapes. Participants are told to press a button to any face or shape display. We will concurrently collect eye-tracking data to measure attention to the eye region of the face image. This is a behavioral task that will also be performed in the MRI scanner. There is no feature of deception. The total time for the task is approximately 20 minutes (5 minutes of training and 15 minutes of task performance).

7.5.1.7.2.25. Mobile App Game: This "mobile app task" is a game style application that assesses the same cognitive functions assessed in the current protocol during visits to the NIMH clinic. However, with the mobile app task, a subset of these functions will be assessed in participants' daily lives, while they are performing a game. Physiological measures, such as heart rate, and behavioral measures, such as reaction

time and behavioral choice, will be monitored during the game using a wristwatch to assess physiology and the same device used for EMA to assess choice. These sets of tasks will allow us to examine the behavioral and physiological aspects of cognition, as they relate to anxiety experienced in individuals' daily lives. The data will be gathered via smart phone technology and will entail both assessment of symptoms and activity, as well as feedback to the participant to potentially augment our ongoing treatment trials. Symptom-based assessments are described on page 18 (see procedures for EMA). The current section describes the procedures for assessing cognition and physiology in tandem with symptoms.

7.5.1.7.2.26 *Approach-avoidance task*: The goal of this task is to present children with an approach-avoidance conflict to measure the differences between avoidance tendencies between children with and without anxiety disorders. In this computer task, children are presented with targets that are paired with 0, 1, 2, 3, 4, or 5 coins (representing money, 'reward') and 0, 1, 2, 3, 4, or 5 lightning bolts (representing noise, 'punishment'). In each trial, the target moves either towards or away from the participant's 'avatar'. If it's moving towards the child, the child can choose to avoid (by moving) or approach (by staying still). If it's moving away from the child, the child can choose to avoid (by staying still) or approach (by moving). If the child avoids the target, there's an 80% chance that nothing happens, 10% chance that the child will get the amount of coins and 10% chance that the child will hear the amount of noises (e.g. 5 noises in a row). If the child approaches the target, there's a 40% chance the child will get the amount of coins a 40% chance that the child will hear the amount of noises and a 20% chance that nothing happens. Rewards are represented by coins, children think they will win more money if they have more coins. Punishments are represented by loud noises (e.g. a scream, a white noise), which the children will hear via headphones. At the end of each trial, the reward or punishment is presented. There are 158 trials in total (around 45 minutes in total), with different ratios of reward and punishment. In this way, it can be computed when children make approach versus avoidance decisions.

7.5.1.7.2.27 *Facial Affect Slot Task (FAST):* This computer-based behavioral task assesses the salience and associations of face emotion cues. The face emotion stimuli are the same type of stimuli included in other tasks in this protocol (e.g., Motion Task, Dwell-Time Task). A comparison condition requires learning contingencies with fruit rather than face stimuli. The FAST task will allow us to examine and computationally model behavioral indices of reinforcement learning in relation to irritability and comparison phenotypes, consistent with the goals of this protocol.

7.5.1.7.2.28 *Ziemba & Goris Confidence Task*: This task has been designed by Ziemba, Goris and collaborators.[169] This task is designed to elicit participants' level of confidence on a perceptual choice. On each trial, participants are asked to categorize a visual stimulus as belonging to category A (for example, rotated clockwise) or B (for example, rotated counterclockwise) and simultaneously report their level of confidence on that choice as high or low. After a short fixation period, four choice targets appear on the screen, corresponding to category A / low confidence, category A / high confidence, category B / low confidence, and category B / high confidence. After 500 ms, the stimulus appears and remains on the screen for another 500 ms before it disappears, indicating that a response must be entered by selecting one of the four targets. The reward structure is such that "high confidence" is a high-risk/high-reward option while "low confidence is a low-risk/low-reward option. Participants accrue reward with each trial in one version of the task. In another version of the task, participants still make the same confidence appraisals in each trial when determining category A and category B but rather than accruing reward during each trial, a random trial is chosen at the end of the task which constitutes their reward. This is a behavioral task that, if it shows a signal, will also be configured for performance while in fMRI, EEG and/or MEG environments. It has no feature of deception. Total compensation will not exceed $30, and participants will be compensated $10 minimum for simply performing the task and up to $20 in additional winnings either added up across all trials or chosen randomly from one trial. The total time for the task is up to 30 minutes. For some subjects, a version of the Ziemba & Goris Confidence Task will be completed in the home. These subjects will view the task on a computer, and the subjects will complete a version of the task suitable for delivery in the home.

7.5.1.7.2.29 *Levy Risk and Ambiguity Task*: This task is modified from the task designed by Levy and collaborators.[170] This task is designed to elicit participants' preferences over known (risk) and unknown (ambiguity) risks. In this task, on each trial, participants choose between a guaranteed gain (or loss) of $5 and a lottery (Figure 2A) In the gain only version of the task, each lottery has two possible outcomes: v or $0, where v ranges from $5 to $50. In the gain/loss version of the task, v ranges from $-50 to -$5 on half of the blocks and from $5 to $50 on the other half of the blocks. Trials are always paired so that participants are choosing between a sure option (either $5 or -$5) and a lottery to potentially gain/lose a larger amount. On half of the trials, v can be received with 1 of 5 known probabilities (p: 13%, 25%, 38%, 50%, or 75%); on the other half, the probability information is occluded and thus incompletely known (the ambiguity context). We will use 3 levels of ambiguity, here termed A: low (24% unknown), medium (50% unknown), and high (74% unknown). Each amount v appears with each p and A level once in random order over 4 blocks of 40 trials (for a total of 160 trials). So-called catch trials offering a guaranteed gain or loss of $5 vs a p chance of $5 will also be included throughout each block. Participants were instructed that each lottery corresponded to a physical bag containing 100 red and blue chips. The bags will always be nearby, and participants can inspect them after the experiment if they wish. For known-risk lotteries, the precise number of red and blue chips in the corresponding bags is specified. For ambiguous lotteries, only partial information is provided, but to eliminate bias, the number of red and blue chips are made equal in the ambiguous displays. To incentivize participants to choose according to their true preferences, participants are given a $50 endowment per session plus (or minus) a variable bonus (or loss). This ensures that if the outcome of the task is a monetary loss, the loss is debited from the endowment and not from the participant's own money. At the conclusion of each session, 1 trial is randomly selected for realization, and the choice made on that trial determines the bonus (+/- $5 or the lottery amount; Figure 2A). No bonus is received for missed responses. This is a behavioral task that will also be performed inside the scanner while functional MRI images are acquired. There is no feature of deception. Total winnings will not exceed $110, and participants will be compensated $10 minimum for simply performing the task and up to $100 in additional winnings. The total time for the task is up to 30 minutes. For some subjects, a version of the Levy Risk and Ambiguity Task will be completed in the home. These subjects will view the task on a computer, and the subjects will complete a version of the task suitable for delivery in the home.

7.5.1.7.2.30 *Subjective Mood Task (SMT):* This task will be conducted while subjects undergo magneoencephalography (MEG) and is designed to monitor subjects' emotional reactivity in relation to past rewards, expectations and environmental settings.[168] In this task, participants are presented with up to 200 trials where they are asked to choose between a certain option and taking a chance. In the chance option, two alternatives will be presented (e.g., win/lose, win/no reward, etc.), which will have different probabilities of occurring. There two trial types: (1) trials where the player is more likely to receive the higher reward/smaller loss (with a ~70% chance); (2) trials where the player is more likely to receive the lower reward/larger loss (with a ~70% chance). Participants are not informed about the trial types. We may display the current earnings only after each block trials, such as after 30 trials to dissociate expectation effects from the impact of outcomes. In a third option the current task earnings may be displayed at all times to exclude a direct relation of happiness to the outcomes, if people were ranking happiness according to outcomes instead of estimating their happiness level. Total possible winnings are no greater than $25 and no less than $2 in addition to $130 for simply performing the task. To monitor emotional response, subjects are presented with a question, "How happy are you at this moment?" after every two to four trials. After a short delay, such as 3 sec, a rating line appears with endpoints labeled "unhappy" and "happy". Subjects will move the cursor along the scale (inducing stress). Prior to each experiment, happiness with one's life overall is measured by asking subjects, "Taken all together, how happy are you with your life these days?". The same question to monitor emotional response is also presented during rest blocks every 10s to 30s. The total time for the task

is approximately 45 minutes. This task includes continuous recording of physiology responses, specifically skin conductance, pupillometry (with eyetracking) and heart rate.

7.5.2. fMRI Task Procedures (optional): Tests of primary study hypotheses rely on fMRI. All tasks conducted in the clinic may be studied using MRI, whereas all tasks studied using MRI may also be used in the clinic. Similarly, all tasks using MRI may be used with magnetoencephalography (see page 32 for a description of procedures).

Healthy volunteers will receive MRI assessments at no more than three points in time. However, depending on scanner availability and subject convenience, the individual MRI sessions at each of these time points may also be divided, conducted over several days. Schedules for these scans will parallel the schedule for patients. The initial MRI will occur after screening interviews and psychological tests. The two follow-up MRI sessions will occur up to 2 years later. Patients will receive MRI assessments at these three time points.

All subjects will be examined with 3.0 T scanners. Depending on scanner availability and subject clinical status, subjects may complete up to six tasks before treatment as well as an additional five tasks during treatment. For patients, tasks may be repeated up to four times per task (both the tasks done prior to and during treatment). For healthy subjects, tasks also may also be repeated up to four times, to provide comparison data for patients.

In the weeks prior to scanning, subjects will undergo a training session in an MRI simulator. During scanning a head support will be used. Subjects will be monitored by a physician or a psychologist. As with healthy subjects, scanning before treatment in patients will be completed in up to three sessions that will not exceed 120 minutes. If scanner time is not available for a full session, the procedures may be divided over more MRI scanning sessions. Scanning after treatment will be completed in one session, during which time two tasks will be completed. In addition, we may obtain a sample of saliva before and after each of the two MRI scans. This will be used to examine associations among salivary cortisol levels, memory performance, and fMRI activation during the emotional memory task. To facilitate scanner calibration, subjects may also undergo four minutes of 10-second epochs of alternating regular breathing and breath holding. As part of this procedure, we may also acquire data while subjects are simply resting comfortably. Another part of the procedure may include a brief (3-4 minute) free-viewing video task, where we acquire data while subjects view a movie. Finally, we also may acquire data on fiber tracts using diffusion tensor imaging (DTI). With each of these imaging procedures, data will be collected as time permits. After the imaging procedures, subjects be asked about the free-viewing task and their responses will be recorded with an audio recorder.

The fMRI studies will be performed to test our main study hypotheses as described below. However, we also are working to develop versions of these paradigms that rely on using real-time fMRI procedures. As such, we eventually hope to implement versions of the paradigms described below in both healthy subjects and patients that provide on-line, real-time feedback of fMRI activity to research participants. However, this will not be possible until considerable pilot work has been completed. This pilot work will also be conducted as part of the current protocol.

The piloting of real-time fMRI procedures will use only healthy adults and healthy juvenile subjects (n= 50). We plan to directly compare task performance in adults and children on the task in an effort to determine the feasibility of studying relatively large samples of both healthy and psychiatrically ill children. Evidence of feasibility would be supported by demonstrating comparable task performance in healthy children and adults. We also plan to evaluate the stability of task performance, with repeated testing, as stable performance also would support feasibility. Therefore, we plan to evaluate each participant's ability to perform the task

procedures on three separate days. The tasks during this procedure will be based on procedures described below, using the same sets of stimuli and similar instructions to subjects. Thus, subjects will be asked to perform movements (e.g. finger tap; button presses), imagine movements, or view images (e.g. facial expressions). In some cases, subjects will be asked to increase or decrease the intensity of the emotional state engaged when they complete these procedures (e.g. increase happiness, decrease fear). fMRI acquisition parameters will be performed within standard guidelines. Depending on the success of these procedures we then hope to eventually conduct the work described below using real-time methods, as will be delineated in later protocol revisions.

7.5.2.1. ***Attention Bias:*** Participants will complete this task both before and after treatment.

7.5.2.1.1. Rationale and Hypotheses: We will contrast brain activity during a task that presents targets within the context of emotional versus non-emotional faces.[19, 27, 28, 66, 67] This task measures attention bias, manifest as a reaction time advantage, to targets that appear proximal to emotional faces. The degree of bias on this task is associated with levels of clinical anxiety.[19, 27, 28] Prior imaging studies rely on words to activate brain areas implicated in attention bias.[54] Rapid word decoding skills may not mature until late adolescence.[64, 75]As a result, using faces in an attention paradigm removes confounds in word-based attention bias tasks between developmental aspects of emotional and linguistic processing. Moreover, prior neuropsychological studies suggest that emotional faces generate larger and more consistent attention bias than threatening words.[19, 27, 28]

Data collected with this paradigm will be used to test four hypotheses. First, in fMRI contrasts of trials with angry versus neutral faces, activations are expected in the amygdala and ventral prefrontal cortex. While prior studies note engagement of the amygdala using emotional faces, this effect primarily occurs with fearful faces. Second, both short and long cued trials will be implemented in this paradigm. Adult studies document enhanced attention bias in both depression and anxiety with long cue presentation but selectively in anxiety with short cue presentation.[19, 27, 28] Therefore, activations in the angry versus neutral contrast are hypothesized to differ in healthy versus both anxious and depressed subjects during long cue presentation but only between healthy and anxious subjects for short cue presentation. These differences are hypothesized in the amygdala and ventral prefrontal region. Third, an attentional bias is expected in reaction time data across angry versus neutral trials, providing a measure of threat bias. The degree of bias is hypothesized to predict the degree of engagement in the limbic regions, particularly ventral prefrontal regions, based on prior data.[21, 28, 53-57, 66, 67] Due to the limited data for this specific task, it is unclear as to whether differences in attentional biases among clinical groups will completely explain hypothesized differences in limbic activation among impaired versus healthy subjects. Finally, effective treatment is hypothesized to alter the degree to which angry faces engage limbic regions and produce threat bias.

7.5.2.1.2. Experimental Paradigm: During this task, subjects respond by right or left sided key-press to indicate the target location (a *-sign). This target will appear in one of two boxes, located on either the right or left side of a fixation cross. Immediately prior to target presentation, visual cues in the form of pictures appear in each box. Picture cues are presented as either neutral or emotionally expressive faces. By comparing reaction time data across various picture cues, a measure of vigilance or attention bias is generated in this task. For the current experiment, one target will be presented during each trial in a 240-trial paradigm. Each trial will last 2000 msec, with an average 1000 msec inter-trial interval (ITI) that randomly varies between 750 and 1250 msec. The experiment is run in four blocks of 60 trials (60 trials x 4 blocks=240 trials), and each block is separated by 12 seconds of fixation. As in Mogg and Bradley, during two blocks, cue stimuli will be presented subliminally, while in the other two blocks they will be presented supraliminally. Between blocks, during fixation, subjects will key-press each time that the fixation blinks.

Each trial consists of a "cue" or presentation slide, consisting of face pairs, followed by a "target" slide. Face pairs are presented in one of three combinations: "neutral" face paired with another "neutral" face ["neutral" trials]; "angry" face paired with a "neutral" face ["angry" trials]; or "happy" face paired with

a “neutral” face [“happy” trials]. Right versus left field-of-view for face presentations is counter-balanced. In 120 trials, supraliminal facial cues will be used, including 40 in each of the pairs listed above. In 120 trials, subliminal cues will be used, with 40 in each of the pairs listed above. For supraliminal trials, emotion and neutral faces are presented continuously for 200 msec. For subliminal trials, emotion faces are presented for 33 msec and are then “masked” with a 167 msec neutral face of that same individual. Blocks of supraliminal and subliminal trials will alternate with fixation.

7.5.2.1.3. fMRI Acquisition: For both the vigilance and emotional memory paradigms, data will be acquired to facilitate event-related analyses. Functional scans will be acquired using a 64 x 64 matrix with echoplanar single shot gradient echo T2* weighting (TR=2000 msec; TE=40 msec; FOV=200mm; 64 x 64 matrix, 3.125 mm voxels) to facilitate an event-related analysis, based on stochastic methods.[75-78]A high-resolution T1-weighted volumetric scan of the whole brain will then be acquired, both for coregistration purposes and to examine morphometry. If time permits, we also will acquire additional data for the purpose of calibration using the above-noted fMRI parameters. This will include data collected during breath-holding and during resting states. Finally, we will also acquire diffusion tensor imaging (DIT) data, using the standard sequence to acquire such data. This will require approximately 15 minutes.

7.5.2.1.4. M/EEG Acquisition: Of note, we also will conduct an examination of brain activation with this task using magnetoencephalography (MEG) with simultaneous electroencephalography (EEG), and we will conduct further studies in the clinic using this task while monitoring eye movements. M/EEG are the only techniques available that directly measures intranueronal currents. Most importantly, they allow for detailed spatial localization of neurological activity with precise temporal information. In this sense, they would allow us to gain a greater understanding of the specific timing of the patterns of neurological activation and processing of information associated with inhibitory and motor functioning. Particular advantages of MEG are that it is generally unaffected by intervening media variations, such as skin, scalp, skull, tissue, fluids, and air, and it requires no measurement referencing. Although EEG is more affected by these concerns, it is more sensitive to brain activity oriented radially to the surface of the head, whereas MEG is more sensitive to brain activity oriented tangential to the head surface. Thus, MEG and EEG each provide unique information which, when combined, provide the ideal complement to fMRI’s excellent spatial but poor temporal resolution. Given their potential value in measuring early sensory cortical functioning, M/EEG have the potential to be a valuable tool for studying psychiatric disorders. In addition, given their noninvasiveness, M/EEG are important and appropriate research techniques for use in children. In adolescents, we will monitor response to the dot-probe using M/EEG three times during the course of treatment. This will include an initial pre-treatment assessment, followed by a mid-treatment and post-treatment assessment.

Collection of MEG data will follow the same methods currently being used in protocol 99-M-0172 in control adults. The research participant will either sit or lie supine in a shielded recording room with their head in a specially designed helmet. Brain magnetic fields will be recorded with the 275-channel OMEGA system. The 275 SQUID sensors are uniformly distributed, in a grid, over the inner surface of the helmet that covers the entire head with provisions for the eyes and ears. Visual and two-way audio communication with the participant will be maintained throughout the session. Head position within the magnetometer will be determined before and after the MEG session by digitizing the position of three indicator coils that are attached to the preauricular and the nasion fiducial points. The positions define the coordinate system for the signals and allow for post-hoc correction of head movement artifacts. Digital photographs of the fiducial points will also be taken to localize the same points on the participant's anatomical MRI scan. MEG is a well-established, non-invasive procedure. Given our success in fMRI data acquisition, we anticipate equal if not greater rates of data acquisition.

During simultaneous EEG recording, participants will wear a stretchy net with 128 sensor sponges soaked in a saline solution on their head while they complete the MEG procedures. Additionally, as part of the EEG process, the researchers will digitize and localize numerous electrodes on the participant’s head

using the Polhemus Fastrak Digitizer—a device consisting of an electromagnetic transmitter and two sensors. One hand-held sensor will be moved over the electrodes to collect information about the locations in 3D space. Participants may wear a small device (like a headband) to secure another sensor to the head. Once the electrodes have been digitized, the sensors will be removed. The digitizing process takes about 10 minutes. The EEG systems described above (Device Name: GEODESICEEG SYSTEM 400 SERIES (GES 400)) are FDA-approved and are being used on label (510(k) Number: K131882).

The MEG systems described above (Device Name: CTF MEG; CTF MEG 151; CTF MEG 275; CTF OMEGA) are FDA approved but have been modified for the purposes of this study. The current MEG techniques use a modified sensor net which is not evaluated by the FDA but does not create additional safety risks. This MEG system, as determined by the NIMH IRB, is considered IDE exempt because it meets the following criteria:

> iii. A diagnostic device (including in vitro diagnostic products) is in compliance with 21 CFR 809.10(c) if the testing:
>
> a. Is non-invasive
> b. Does not require an invasive sampling procedure that presents significant risk
> c. Does not by design or intention introduce energy into a subject, and
> d. Is not used as a diagnostic procedure without confirmation of the diagnosis by another, medically established diagnostic product or procedure.

The MEG system described above meets each of the category III criteria: (a) it is non-invasive; (b) it does not require an invasive sampling procedure that presents significant risk; (c) it does not by design or intention introduce energy into a subject; and (d) it is not used as a diagnostic procedure without confirmation of the diagnosis by another, medically established diagnostic product or procedure.

7.5.2.1.5. Eye-movement: We will also collect eye movement data during this task implemented in the clinic, both in a well-lit and darkened room, to examine the effects of ambient light on ocular activity. Finally, we will also complete a memory test using pictures of objects and faces to further examine eye-scanning patterns during face and object viewing. Subjects will complete 14 computerized encoding-recognition blocks that alternate between various types of simuli. In 10 of these blocks, neutral faces and inanimate control stimuli (electric fans) are presented in alternating blocks. In the other four blocks, scenarios used in prior research are presented depicting intentional versus accidental actions that caused harm to people or objects. [101, 102] These scenarios have been shown to elicit empathic responses in children as young as four years old. We will be utilizing an identical eye tracker to that of the Saccade Task (above) with the exception of utilizing a chin-rest to minimize head movement. Procedures will be identical to those described above. However, brain activation will be quantified using MEG, or, for behavioral studies in the clinic, only eye scanning patterns will be examined.

7.5.2.1.6. fMRI Study of Attention Retraining: We will collect imaging data during attention retraining in a group of healthy adults with high scores on anxiety symptom scales (e.g., STAI, SAS). This project will involve in-scanner administration of the attention-orienting task described above as a pre and post-measure of attention bias as well as an initial attention retraining session, conducted in the scanner. In attention retraining, subjects will complete dot-probe threat-neutral and neutral-neutral face pairs. Half of the sample will receive “active” attention retraining, which is expected to bias attention away from threat (e.g., anger or disgust faces). The target probe will appear at the location of the neutral face 100% of the trials.

Half of the sample will receive "sham" retraining, which is not expected to bias attention towards or away from threat. The target probe will appear at either location with equal probability.

Subjects willing to continue will also receive behavioral attention retraining twice a week for 4 weeks at the University of Maryland in Nathan Fox's laboratory or at the NIH, using a shortened version of the training. All subjects will return to NIH for a subsequent fMRI scan using the attention-orienting task.

We expect "active" attention retraining will result in neural changes and reduce stress responding. We would like to see if this helps subjects pay attention in both the games and in other parts of their lives. To test the effects of training on stress-reactivity will be completed outside of the scanner following initial training and long-term training. These tasks have several elements that subjects may find mildly stressing. In the difficult and unsolvable anagram task, subjects will be asked to solve as many words as possible within 3 minutes and told that their performance is being monitored. The subject will not know that most of the words are unsolvable. Following the test, the subject will be told that their performance was unusually low. Mood ratings will measure stress reactivity to this task. In the dyadic interaction task, the subject will be placed with an unfamiliar peer (confederate). The participant and the confederate will be placed in a room together for five minutes. In another stressor task, the participant will be asked to prepare a speech, then deliver the speech and count backwards from a high number by 3's and their performance will be monitored by a small audience. These stress tasks may be videotaped for later behavioral coding. After the procedures are complete, the subject will be told of the deception used in order to provide a mildly stressful situation.

7.5.2.2. *Emotional Memory:* This set of tasks will be completed before treatment by adult subjects and both before and after treatment by juvenile subjects. These tasks will use three sets of stimuli: static faces, brief film clips depicting faces, and static emotionally-evocative pictures. Depending on subject fatigue and availability of scan time, tasks employing the three sets of stimuli may be completed in a single scanning session or across multiple scanning sessions. Of note, the structure of the task will be the same for each scanning session. However, each viewing of the task only will rely on stimuli drawn from one of the three classes of stimuli (i.e., static faces, film clips of faces, evocative IAPS pictures).

7.5.2.2.1. Rationale and Hypotheses*:* Prior studies briefly reviewed above document associations between anxiety or depressive disorders and emotional modulation of declarative memory. Prior imaging studies in healthy adults document an effect of emotional valence on the engagement of cortico-limbic brain regions.[55, 64, 79] The current set of memory paradigms is based on such research. The paradigms systematically vary emotional content of to-be-remembered stimuli. At encoding, while fMRI data are acquired, subjects will view emotionally expressive faces, brief film clips of such faces, standard emotional IAPS photographs, and symbols that predict the forthcoming presentation of these stimuli. Memory for these stimuli will be probed approximately 20 minutes later, outside of the MRI scanner. Encoding will occur while subjects rate emotional and physical attributes of stimuli. Prior imaging studies find this procedure to engage limbic regions, including ventral frontal regions.[79-81]

Data collected with this paradigm will be used to test four hypotheses. First, as among adults,[80, 81] the contrast of emotion with physical attribute ratings is hypothesized to engage limbic brain regions, particularly the ventral inferior frontal cortices. Second, such activations are hypothesized to differ in impaired versus healthy subjects. Third, viewing emotional stimuli and cues that predict their forthcoming presentation is hypothesized to engage limbic regions, relative to viewing stimuli of neutral valence or cues that predict forthcoming presentation of such stimuli. This effect is expected to differ among healthy and impaired groups. Based on available data,[21, 23-26, 29, 53-57, 61, 68, 70] it remains unclear the degree to which differential engagement will be seen during viewing of negative or positive stimuli contrasted with neutral stimuli.[21, 56, 57, 80] Fourth, memory for previously rated stimuli is known to be influenced by the emotional reactions to the stimuli, the degree of fMRI activation within the amygdala, as well as the degree of clinical anxiety or depression.[22, 55, 80] As a result, strength of amygdala activation during encoding is hypothesized to correlate with both subjective ratings during scanning as well as the degree of recall advantage after scanning

for emotionally evocative stimuli. Strength of hippocampal activation, in contrast, is hypothesized to predict overall accuracy in recall, independent of any bias for emotionally evocative pictures.

7.5.2.2.2. Experimental Paradigm: During this task, subjects will view a series of 24 different facial photographs of 24 different individuals, 24 different IAPS pictures, or a three-to-four-minute cartoon film clip depicting a social interaction that unfolds in the movie. Different photographs will be used in the emotional memory and attention paradigms, as well as with repeated administrations of the emotional memory paradigm. We limit the total stimulus set at each administration to tailor the stimulus set to the memory demands of the paradigm. Prior studies demonstrate optimal memory performance (approximately 60-70% correct) using the behavioral parameters employed in the current task. Facial photographs have been drawn from standardized emotional expression data sets, and faces express "happy", "afraid", "angry", or "neutral" expression (6 of each expression). Similarly, the 24 IAPS pictures have been drawn from standardized data sets, and the pictures systematically vary in emotional content. Finally, there are three videos The first video was drawn from publically-available data sets. The first video has been employed in a study where the data were analyzed by Drs. Finn and Bandettini, collaborators on this protocol, among more than 200 youth in the age range in the current protocol. As in prior studies, pictures and videos will be selected from three categories, including: i) negative valence pictures or scenes, ii) neutral pictures or scenes, and iii) positive valence pictures or scenes. In the video, valence will be conveyed by the facial expressions and other cues displayed by the individuals depicted in the clips (see first film clip: https://vimeo.com/152985022).

The second video was selected because the video is specifically relevant for anxious youth and is intended to elicit anxiety. We will require that all parents view the video before showing it to their children. Parents will be shown the video at the consent visits, parents will determine if the video is appropriate for their child, and we will document their choice regarding the video via a checkbox on the consent form. Parents will be told that their child cannot view the video before the MRI scan. We will monitor children's reaction to the video carefully during the study (see second film clip: https://vimeo.com/278734339 (password: NIMH1234).

The third video (a clip taken from a short animated film, "*Sintel*") was selected because the video is specifically relevant for anxious and depressed youth and is intended to elicit sadness. The purpose of this clip is to study mood induction, which is severely constrained if we cannot induce negative mood. For negative mood induction, the film clip is designed to induce mild anxiety or sadness. Similar mood induction film clips were previously approved by the IRB in our 18-M-0037 protocol. As with previously approved clips, these films are rated PG or PG-13 and do not depict sex, gore or violence. Parents will be shown the video at the consent visits, parents will determine if the video is appropriate for their child, and we will document their choice regarding the video via a checkbox on the consent form. Parents will be told that their child cannot view the video before the MRI scan. We will monitor children's reaction to the video carefully during the study (see film here: https://www.youtube.com/watch?v=1wVrTwoUFPI).

For faces and pictures, stimuli will be presented three times, where different ratings will be made. In one epoch, subjects will rate an external feature of the face, picture, or scene. For a second epoch, subjects will rate the degree to which the face or picture depicts a negative scenario, such as a hostile emotion for a face or a dangerous situation for a picture. In prior neuropsychological studies, anxious subjects show a mnemonic bias for faces rated as hostile.[22]In a third epoch, subjects will rate the degree to which they feel anxious upon viewing the face, picture, or scene. For the film clip, memory will be assessed through an interview with the subject, which will be recorded and transcribed, to determine the salient features that are attended and remembered.

7.5.2.2.3. fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.3. *Social Interaction Tasks*: Most of these tasks only will be completed before treatment. However, one social interaction task may be completed twice. In patients, this will involve completing the task both before and after treatment.

7.5.2.3.1. Rationale and Hypotheses: Social interactions are a prominent source of concern for individuals with mood and anxiety disorders. Observational studies have shown that during such interactions, anxious and depressed adults show heightened self-protective behaviors, including avoidance, submission, and lack of self-assertion. Similar patterns of behavior in conflictual or evaluative social circumstances have been observed in anxious and depressed youth. Although these disorder-linked behavioral patterns are well-documented, relatively little is known about their association across development with activation in disorder-relevant neural structures, including the amygdala and orbitofrontal cortex.

To address this gap, it is necessary to adapt social interaction paradigms for use in an imaging context. Each task in this protocol permits ecologically valid simulation of social interactions, thereby facilitating examination of disorder-associated perturbations in social behavior and their neural correlates. Both tasks are used to test the hypothesis that adolescents with anxiety disorders and major depression will exhibit enhanced activation in the amygdala and ventral prefrontal cortex during episodes of social rejection.

7.5.2.3.2. Experimental Paradigms: Two tasks will be completed. Both involve deception. Moreover, subjects may complete one of these tasks on two occasions. Subjects will be debriefed after completing the two tasks, and, if the subject completes one of these tasks on two occasions, they will be debriefed when completed the second task on the second occasion. For subjects who are children, both children and parents will be told about the deception.

7.5.2.3.2.1. The first task examines changes in the salience of social relationships, particularly opposite-gender relationships. These changes exert potent influences on risk for psychopathology, particularly major depression, possibly through effects on motivational state and self esteem. Indeed, stress stemming from interactions with the opposite gender represents one of the most potent longitudinal risk factors for major depression. Among adults, a few fMRI studies have begun to examine aspects of opposite-gender evaluation, but no such studies have been conducted with adolescents. The current behavioral paradigm is designed to begin the process of developing such a task. This task involves deception. Subjects are told that they will be evaluated by peers, but this does not occur. Subjects will be thoroughly debriefed about the task and the deception involved. The task consists of three phases: in phase-1, subjects rate the social desirability of photographs of same- and opposite-gender peers; subjects are also photographed with a digital camera, and the subject is told that their picture will be rated over the internet by peers. In phase-2, subjects rate how desirable the subject feels he or she might be to peers in the photographs; in phase-3, subjects assess their mood after they are told that subjects had viewed their pictures over the inter-net and received a “yes” or “no” answers concerning interest to meet the subject. Only phase 2 and phase 3 are performed during neuroimaging (fMRI, M/EEG).

7.5.2.3.2.2. The second task expands on the process of examining social interactions initiated in the first task; both tasks assess neural responding to anxiety-provoking peer interactions. However, this second task initiates interactions using a virtual computer-generated environment, as opposed to photographs. Thus, this second task assesses how anxiety is influenced by accepting and rejecting personas in a more interactive context such as might be encountered in online interactions. This new task also allows minimal structured responses to peer statements. This virtual interaction task involves deception. Subjects are told that they will evaluate, and be evaluated, by peers with whom they interact during a computer game. In reality, no such interactions occur. Subjects will be debriefed at the same time that they are debriefed concerning the other deception. The task consists of two phases. The first phase is completed in the clinic. The second phase is completed during neuroimaging (fMRI, M/EEG). Thus, in **phase-1**, the subject uses a computer program to generate and name a cartoon avatar, which serves as a graphical representation of the research participant, as it will be represented in a computer interaction game. The subject also answers questions about their likes and dislikes. The subject is told that prior to the game, peers will review their avatar and their likes and

dislikes. The subject then sees 6 cartoon avatars that represent different types of peers with whom the research participant will purportedly interact with in the game. Prior to scanning, the subject will also read short descriptions about those peers and will be asked to rate their desire to interact with each one. In **phase-**2 during the game while neuroimaging is taking place, the subject enters a series of virtual "rooms" populated by these avatars. In each room the peer avatars will make positive, negative, and neutral comments to the subject, and the subject will have the opportunity to respond to those comments. For example a "nice" peer might say "I like your avatar" whereas a mean peer might say "I think your avatar is lame". After the interaction game is over, the subject will rate how much they enjoyed interacting with each of the other peer avatars. Phase 3 will occur immediately after neuroimaging. In this phase, subjects will be re-introduced to the virtual environment and will be asked to select a seat in the environment where they will become part of the virtual classroom. A series of questions about the reasons for their choice will be probed.

7.5.2.3.2. fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.4. *Fear Conditioning:* As described above, children and adults will undergo fear conditioning procedures in the psychophysiology laboratory or during neuroimaging (fMRI, M/EEG). Moreover, some subjects may complete fear conditioning procedures in the psychophysiology laboratory and then undergo a subsequent procedure in the scanning suite at a later date. In addition, this neuroimaging may be repeated up to 2 years later.

7.5.2.4.1. Rationale: The rationale for the fear conditioning procedures is provided on page 13-14.

7.5.2.4.2. Experimental paradigm: A four-minute baseline is acquired followed by a habituation, preacquisition, acquisition, and extinction phase. During the preacquisition phase, participants are presented with 2 neutral stimuli (i.e., pictures of 2 adult female faces; circles of different sizes). They view 4 pictures each of the two different female faces, totaling 8 presentations, or they might view similar presentations of different-sized circles corresponding to the CS+ and CS-. Following habituation, participants view these same two female faces or same circles, but one face is paired with a moderately aversive stimulus (i.e., a loud 95 dB scream, the unconditioned stimulus [UCS]) over 8 trials while the other stimulus is not paired with an aversive cue. This is followed by an extinction phase, which 12 presentations of each cue without the UCS. This task will also be competed a second time, using morphed facial or circle-based presentations of CS stimuli. This second version of the task will be completed at a later date, as part of the follow-up study. Both aspects of this protocol may occur either in the scanning suite or in the psychophysiology laboratory, as noted above.

7.5.2.4.3. fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.5. *Eye Movement Reward Task:* Eye movement procedures will be implemented both behaviorally in the clinic and in the MRI scanner.

7.5.2.5.1. Rationale: The rationale for the eye movement task procedures is provided on page 16.

7.5.2.5.2. Experimental paradigm: Paradigm for the eye movement task is provided on page 17.

7.5.2.5.3. fMRI and M/EEGAcquisition: Eye movements are measured using a PC that serves as a user interface and a digital data recording device; a camera that captures eye movements; two video monitors, one for displaying an image of the eye, and one for displaying the scene that the subject views (with a set of cross hairs superimposed indicating the subject's point of gaze); a magnetic head tracker providing information about head position, that consists of a control unit, a transmitter module, and a small sensor attached to a baseball hat worn by the subject; and finally a model 5000 eye tracking control unit, which contains the processing board that integrates and coordinates all of the above-mentioned components. The eye tracking system is complemented by a second PC (the task PC), on which study subjects view

stimuli and complete tasks. The camera measures pupil diameter and eye movements by capturing the eye's back-projection of a beam emitted by a ring of LEDs placed around the camera's lens.

Eye tracking is non-invasive and painless. Before starting the task, each subject needs to complete a calibration procedure to account for individual differences in eye physiology and task set-up. During this calibration procedure, the subject is required to look at each of nine points displayed on the task PC. This procedure can take – depending on the subject – anywhere from 1 minute to several minutes. During the task, subjects can move their heads within a small range, but are nevertheless prompted to move as little as possible. A chair specially designed to minimize movement and to support the subject head's is provided. Eye tracking parameters are reaction time (first fixation after onset of a stimulus), accuracy (eye position relative to a target stimulus), number of fixations after stimulus onset, and velocity of eye movement.

7.5.2.6. *Reward Tasks:* As noted above, we will administer various reward tasks in the clinic or scanner, including the Monetary Incentive Delay Task (MID) or other reward tasks. These tasks will be used both behaviorally and with fMRI. Of note, we will administer as many as six fMRI reward tasks.

7.5.2.6.1 Rationale: The rationale for task procedures is provided on page 18.

7.5.2.6.2 Experimental paradigm: The experimental paradigms appears on page 24 and page 29, where the instrumental-learning task is described, including creward-related events. As described on page 29, the instrumental learning task simulates carnival games and has multiple phases. Stimuli used during imaging are described here. In one phase, participants will view static, colored images of objects (such as bells or balloons). The images will be different (e.g., colors), and will switch their location on the screen randomly. Participants will have two hand grip response devices in each hand and will grip one of the devices while the images are on the screen to select one image. After participants respond, the screen will acknowledge their selection by showing an image of a mallet overlaid on one image. Next, a feedback screen will indicate whether participants' choice is rewarded, such as "Win 50¢!" or "No Win." During this phase, participants learn to select the image that is followed more frequently by reward. In another phase, participants will view two static, colored images on the screen. For some percentage of the participants' correct responses, the selection will be followed by a reward (such as "Win 50¢!"). For the other correct responses, feedback will be rigged, which will be followed by no reward (such as "No Win"). Incorrect responses will be followed by no reward (such as "No Win").

7.5.2.6.3 fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.7. *Conflict Adaptation Task:* Participants are presented with stimuli that combine fearful or happy faces with the word "FEAR" or "HAPPY" written across the face. Participants are asked to indicate the emotion of the face, while ignoring the distracting effect of the written word. Additional blocks present other, non-emotional words as control trials. This task, which requires 15 minutes to complete, examines how subjects monitor and adapt to conflict from trial to trial. These cognitive adjustments, critical in daily life, are automatic. We are interested in testing whether these automatic processes differ in anxious individuals, and potentially contribute to the attention bias characteristic in anxiety disorders. This task will be used both behaviorally and with neuroimaging (fMRI and M/EEG).

7.5.2.7.1. Rationale: The rationale for task procedures is provided on page 16.

7.5.2.7.2. Experimental paradigm: The experimental paradigm appears on pages 16.

7.5.2.7.3. fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.8. Affective Posner Task: As noted above, we will examine the interaction between attention and emotion using an established task.

7.5.2.8.1. Rationale: The rationale for task procedures is provided on page 22.
7.5.2.8.2. Experimental paradigm: The experimental paradigm appears on pages 22.
7.5.2.8.3. fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.9. *Emotion Control Tasks:* Three different emotion control tasks, described above (see pp. 14-15), rely on IAPS photographs to engage emotional processes while examining interactions between these emotional processes and aspects of cognitive control. One of these tasks, The Emotional Number Stroop (see page 15), requires participants to indicate which of two serially presented stimuli, bracketed by IAPS pictures, contain more numerals. The second, The Emotional Interrupt (see page 15), requires participants to identify a geometric target, which is bracketed by IAPS pictures. The third, the Emotional Regulation Task (see page 18), requires subjects to up or down-regulate their emotional responses to IAPS pictures.
7.5.2.9.1. Rationale: The rationale for task procedures is provided on pages 14-15 and 18-19.
7.5.2.9.2. Experimental paradigm: The experimental paradigms appear on pages 15 and 18.
7.5.2.9.3. fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.10 Inhibitory Control Tasks

7.5.2.10.1. AXCPT Task: this is a version of the task that has been used in the clinic, as described on page 20. This task is one component of the CPT trilogy task, described in more detail in this section, as used during fMRI and M/EEG. To measure proactive control, we will use the AX-CPT task in the scanner.[173,174] The AX-CPT presents a continuous series of letter pairs (i.e., a cue letter followed by a probe letter) dissociated into 4 trial types: AX, AY, BX, and BY. AX trials were the target trials, meaning that when participants saw an "A" cue followed by an "X" probe, they were to press a different button in response to the probe than during the other 3 trial types. Specifically, participants were instructed to press a nontarget button (e.g., "1") following every cue, and following most probe types; however, if an "A" cue was followed by an "X" probe, participants were to press a target button (e.g., "4") upon seeing the probe. In our version of AX-CPT, AX trials will be the majority of the time and the other trial types (i.e., AY, BX, and BY) will each be presented infrequently (Troller-Renfree et al., 2019). Participants will complete a total of 400 trials in random order, in a task that will require 30 minutes to complete. Each trial will begin with a center fixation cross appearing for 200 ms, followed by the presentation of the cue stimulus and finally the probe stimulus.[175] For some subjects, a version of the AXCPT Task will be completed in the home. These subjects will view the task on a computer, and the subjects will complete a version of the task suitable for delivery in the home.

7.5.2.10.1.1. fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.10.2. Stop/Change Task: This is a well-established paradigm to assess cognitive flexibility. Previous research using this task in healthy adults has demonstrated that cognitive flexibility during this task is associated with recruitment of ventral medial prefrontal cortex, dorsolateral prefrontal cortex, parietal cortex, and striatum.[171] This is a version of the task that has been used in the clinic, as described on page 20. This task is utilizes procedures from the stop task, aadministered in the clinic, that is described in more detail in this section, as used during fMRI and M/EEG. However, little is known regarding the extent to which cognitive flexibility is impacted by emotional dysregulation such as feelings of frustration and the neural mechanisms behind it. Results from this assessment will demonstrate whether frustration negatively impacts one's ability to flexibly execute alternate response options. We will use an adapted Change Signal Task to study cognitive

flexibility under high-frustration vs. low-frustration conditions. Frustration is induced by manipulating the timing of the stimuli to make correct responding challenging and by providing participants rigged feedback.

Participants are given a computerized Change Signal Task.[172] During this task, participants will be presented with a cue (arrow pointing left or right) which indicates the button they should press (e.g., left pointing arrow = press with left hand). On a small proportion of trials, the subject will be presented with a "change cue" at some point during the response window, which will instruct them to press the button opposite to the original cue instruction (e.g. Change cue: right point arrow = subject must shift from pressing with left hand to pressing with right hand). The change cue will be presented at various points during the delay in order to create "easy" versus "hard" trials. This task will allow examination of the ability to inhibit inappropriate responses and execute alternate responses under high-frustration vs. low-frustration conditions. During high-frustration conditions, the task is programmed such that the participant's error rate is approximately 50% and the participant receives rigged feedback on 20% of the trials. During low-frustration conditions, the task is programmed such that the participant's error rate is about 10% and the participant only receives accurate feedback. Participants are instructed that they will receive monetary compensation for completing the task. All participants start with $50 and are instructed that they can lose $0.50 for each incorrect answer. In the end, participants receive $75, in addition to their compensation for completing this task, regardless of their task performance and the amount they win during the task.

The whole task, including instructions, lasts for about 40 minutes. Immediately after completion of the task, participants are debriefed that we programed the computer to provide rigged feedback in some trials. In addition, during consent/assent, participants are told that some tasks or games may have misleading information. They are told they can elect not to participate in tasks/games with misleading information if they tell us they are opting out of any such games. Of course, as with all our tasks, participants may stop the task at any time. During debriefing, we explain that the misleading information was necessary to study how people react to frustration and how their attention and cognitive ability is influenced by the feelings of frustration. For some subjects, a version of the Stop/Change Task will be completed in the home. These subjects will view the task on a computer, and the subjects will complete a version of the task suitable for delivery in the home.

7.5.2.10.2.1. fMRI and M/EEG Acquisition: Parameters will be identical to those used in the attention paradigm.

7.5.2.10.3. Mobile Version of Inhibitory Control Tasks: We seek to develop a mobile version of the AXCPT and Stop/Change tasks, that will parallel the lab version structurally. The aim is to collect data on inhibitory control performance in a naturalistic setting and learn about these functions in daily lives. Participants will download a smartphone application for the task either on their personal smartphone or on a password-encrypted mobile device provided by the NIMH and will complete the task either in the lab or *in vivo*, or both. All behavioral data collected will be deidentified and will not be stored on the mobile device. Subject data will be extracted to Firebase, a cloud service that is managed by the NIMH; each subjects' data will be associated with a unique five-digit identification code and exported as a comma-separated value (CSV) data file. Ultimately, the remote nature of inhibitory control application has the potential to make data collection easier, for both researchers and participants, lead to more diverse samples, and increase the ecological validity of inhibitory control research.

7.5.3. Treatment (optional):

7.5.3.1. *Overview:* Youth can choose open treatment with either fluoxetine or psychotherapy. Adults can only receive open treatment with fluoxetine.

For subjects receiving fluoxetine, treatment procedures follow those from prior studies in pediatric mood and anxiety disorders. Potential subjects undergo a baseline assessment with the K-SADS.[63] Patients will be allowed to continue contact with outside (non-NIMH) health care professionals throughout their participation, as long as no other treatments are initiated. Supportive psycho-educational therapy is initiated, following guidelines developed by our group and implemented in prior trials. Subjects are then followed for three weeks, at which time anxiety and depression are reassessed via K-SADS. Subjects who continue to meet study criteria will be offered treatment. For those patients who elect to initiate treatment, patients may have between three and 12 visits as part of the treatment research component of the study. Subjects may receive additional treatment visits, as they are transitioned to the community. Subjects who do not continue to manifest symptoms are followed for one month. Of note, due to limitations in the availability of clinicians, adults entering this protocol will only be eligible to receive open treatment with fluoxetine, studied and treated by Dr. Pine's group directly. Similarly, due to limitations in availability of scanner time, adults will only be scanned once, prior to treatment.

For subjects not receiving medication, treatment is delivered by a psychologist using a standardized psychotherapy regimen. For treatment of anxiety disorders, cognitive behavioral psychotherapy (CBT) is used. Procedures follow the CBT manuals developed by Dr. Wendy Silverman who is involved in the current protocol. Thus, subjects are eligible to participate even if they refuse medication treatment. Treatment choice is based on a comprehensive review of all relevant clinical issues with the patient's family. After this review, the child may receive treatment with fluoxetine or with individual cognitive behavioral therapy. In either instance, subjects will complete all other aspects of the study completed by subjects in the blinded treatment phase. Thus, subjects may undergo weekly visits, as listed below, and they may receive both fMRI and neuropsychological assessments both before and after the eight-week treatment study.

Due to the COVID-19 pandemic, for those potential participants who live within a 50-mile radius from the NIH, we will be offering the option to engage in research visits/encounters in-person or via telehealth, when deemed appropriate and safe for the participant. For those participants who indicate a preference to participate in research via telehealth, we will offer to conduct the informed consent visit and the cognitive behavioral therapy sessions via telehealth.

The patient care and research activities provided via telehealth will meet the same safety and quality standards required during in-person clinical encounters and will comply with all appropriate policy statements, regulatory requirements, and accreditation standards.

Not all patients may be appropriate candidates for telehealth encounters involving cognitive behavioral therapy sessions, and for those participants telehealth encounters will not be an option. Factors that help determine this are: any safety issues, participant's history with compliance and success with past telehealth therapy, and attendance and success with past virtual encounters or virtual learning. If clinicians have a question about whether telehealth can successfully meet the standards of quality and safety for a particular patient or type of encounter, the clinician will confer with the Principal Investigator, Dr. Daniel Pine, to determine appropriateness of this modality for the patient. Telehealth capabilities will not alter the practice of subject safety monitoring as detailed in this protocol. Clinicians have established a process for escalating to a higher level of care in advance of the encounter, including establishing the physical location of the patient and family in advance of the encounter and an alternate contact number in case the encounter is disconnected.

The physical environment/space in which the telehealth encounters will occur will be large enough to accommodate comfortable seating for the clinician as well as a work surface with the equipment necessary to complete the visit. The space will be private, with a door to prevent interruptions during a telehealth encounter. The space will have appropriate and adequate lighting, be uncluttered, contain no documents or other items with Personal Identifiable Information (PII) or other confidential information within the view of the participant, and have no background images/objects that might be distracting to the participant. The

participant will be instructed to conduct the encounter in a similarly private environment, with adequate lighting, free of distracting noises, and in a way that minimizes interruptions.

We will comply with the clinical center's policy (MAS policy M20-1 Utilization of Telehealth/Telemedicine by NIH Healthcare Providers for NIH Clinical Center Patients) for NIH investigators who conduct their research at the NIH Clinical Center and will utilize Microsoft Teams for the conduct of synchronous telehealth visits/encounters between clinician and their patients. Microsoft Teams is cloud-based team collaboration software that is part of the Microsoft® Office 365 suite of applications, which is operated and managed for NIH by the Center for Information Technology (CIT). Microsoft Teams does not store any aspect of the visit; no recording, sharing, storage of data, files or video will be provided. Both clinician and patient will only use teams as scheduled and allowed by the clinical center.

Telemedicine appointments will be scheduled by entering a "Virtual Visit Electronic Appointment Request" in CRIS, preferably no less than 24 hours in advance. Once entered, arrangements will be coordinated by the CC Telehealth Concierge Service with the patient and then added to the clinician's MS Teams-Outlook calendar.

For youth receiving treatment of an anxiety disorder, CBT will be randomly assigned to receive one of two forms of computer-based attention-retraining therapy. The active form of this treatment is designed to augment principles used in the extinction of anxiety, as most directly employed in CBT. The augmentation treatment extends recent work[82] that demonstrates benefit in adults and children. Finally, some children and their families may not be willing to be treated with fluoxetine and may be poor candidates for the intensive form of CBT, which requires weekly visits to NIH and exposure sessions at the NIH.

In the past, we have found that more than 80% of all children who request any form of treatment as part of the current protocol can be successfully treated with either CBT or an antidepressant. For other children needing treatment who cannot receive CBT or an antidepressant, a less intensive form of CBT will also be offered. Children receiving this less intensive form will not be included in the analysis of data from the randomized controlled trial (RCT), which will only include children receiving more intensive form of CBT. Moreover, as this other treatment option is less intensive and less established than the other two treatment options, this third treatment will only be offered to children who cannot tolerate the more intensive form of CBT and who are not candidates for antidepressant medication treatment. Finally, families will be told that this form of treatment is less established than either of the other two treatments but is available to families who are not candidates for either of the other two treatments. Again, this is expected to be a small number of all participants seeking treatment. This manualized treatment also was developed by Dr. Silverman. The treatment is known as Supportive Parenting for Anxious Childhood Emotions ("SPACE").[151] The treatment uses the same procedures and the same general outline as the other form of CBT to be used in the trial. However, SPACE involves weekly visits with only a parent, who is taught procedures to be used with their child, as well as visits with the child at less frequent intervals. Since the standard form of individualized CBT is the more established and more intensive form of treatment, SPACE only will be offered to families who desire CBT but cannot conform to the requirements of the standard form of CBT.

Of note published research on computer-based attention training tests efficacy as a stand-alone therapy. The current protocol examines augmentation of response to established treatments. The most successful attention-training interventions have trained anxious individuals to shift their attention away from threats. In adults, this training has been given irrespective of threat bias levels on study entry. However, in the absence of any other concurrent treatment, such as CBT or SSRIs, there has been concern that training children to avoid threat could serve to sustain anxiety, unless children entered a trial with a pre-existing bias to monitor threat. This concern reflects the fact that a bias away from threat, much like a bias towards threat, has been shown to predict anxiety in traumatized adults[100]. This might be because bias away from threat is a form of threat avoidance, and other forms of threat avoidance predict persistence of anxiety. These findings informed the only prior study that trained anxious children to avoid threats[99]. In this prior study, training only was provided to children who entered the study with a bias towards threat, so that the training would correct a pre-treatment bias towards threat. Training children who enter a study without a bias towards threat could induce attentional threat avoidance. Of note, no data in children demonstrate harmful effects of such attentional-threat avoidance.

Nevertheless, on theoretical grounds and based on observational data in adults, such avoidance might potentially interfere with CBT.

In the initial phase of the current protocol, 30 children were randomly allocated to receive training in directing their attention to happy cues. This training procedure generated no evidence of efficacy, and there is a need to revise the training procedures. In another, recently published study of attention-retraining with anxious children,99 participants were trained to avoid threat. In this second study, only children with a preexisting bias received training; children without an attention bias were not enrolled, due to concerns about adverse effects in children receiving no other treatment. This study demonstrated efficacy of this training regimen, delivered as a stand-alone treatment. In the second phase of the current study, we used these same training procedures, delivered over eight weeks, in tandem with CBT. These procedures were completed in 75 children, approximately half of whom received the active form of attention-retraining (attention bias modification treatment [ABMT]) and half of whom received the placebo training. The analysis of these data showed a substantial benefit on anxiety symptoms in children and adolescents with an anxiety disorder receiving Cognitive Behavioral Therapy (CBT). However, evidence of differential efficacy of CBT was equivocal; a consistent pattern of differences did not emerge between subjects receiving active and placebo ABMT. Of note, we have found that effects of ABMT can vary based on participant engagement in the training, as quantified with a reaction-time (RT) measure and that individuals with anxiety tend to show less engagement than individuals without anxiety based on such RT-based measures (see Abend et al. 2013). For subjects in the current protocol, we did find that our RT-based measure of training did not differ among subjects randomized to the two treatment conditions. This suggests that our failure to demonstrate an enhanced effect of active relative to placebo ABMT could reflect insufficient engagement in ABMT. In the third phase of this protocol, the hope is that with better engagement, we can find a beneficial effect in a subsequent trial of 120 subjects. Before beginning the next randomized controlled phase of this protocol, we piloted the procedures for active ABMT, to make sure that we can achieve acceptable levels of training, as quantified by our reaction-time-based measure. Ten participants completed the ABMT pilot. As a group, these participants showed good accuracy on the behavioral paradigm (mean = 86.29%) and performed at an expected speed (mean reaction time = 492.81 ms). This demonstrates acceptable data to move forward with the randomized phase of the training experiment. Change in reaction time was examined across the blocks of the task to examine online learning. Eight of the ten participants showed improvements in reaction time through online training, which was statistically significant even in this small sample (mean improvement = 28.43ms, $t = 3.07$, $p = .009$). This is superior relative to our prior procedure, which again demonstrates acceptable data to move forward with the randomized phase. Participants will be randomized into active and placebo conditions, and the procedures for the trial will be identical to phase two.

The attention-retraining therapy in this third phase is embedded in the context of a neutral video game. This involves the same attention bias paradigm described above. In brief, this paradigm presents a neutral "dot-probe" target in one of two locations, which is immediately preceded by the presentation of two faces. In each retraining session that will occur at each treatment visit to the NIH, children will receive approximately 160 angry-neutral dot-probe discrimination trials of their designated training condition (approximately 10 minutes). In the attention-retraining condition, participants will view Angry-Neutral stimulus pairs with the target probe appearing at the location opposite of the angry face (in the location of the neutral face) for 100% of trials. In the exposure-only condition, participants will complete the attention bias paradigm with the target probe appearing at either location with equal probability . Thus, subjects will complete weekly training sessions on-site during their regularly scheduled appointments throughout an 8-week treatment. Testing sessions with all face pair combinations (i.e., Angry-Neutral, Happy-Neutral and Neutral-Neutral face pairs) may be administered during treatment sessions occurring at 1,2,4,6 and 8 weeks to monitor attention biases. For both the active and neutral versions of the task, the task will be delivered as part of a videogame, with the hope of increasing subject engagement and increasing the magnitude of training in the active ABMT condition, as reflect in reaction time.

We may use material incentives to encourage subjects in our study to complete all of their attention retraining sessions. As when incentives are offered in standard Cognitive-Behavioral Treatment (CBT) with children and adolescents, in our study incentives will motivate participants to complete crucial treatment assignments. The patient's therapist in concert with the patient's parent(s), will tailor the incentives to each

individual. The selection of incentives will depend on the age and interest of the individual and include items such as "Webkins", a movie pass, or an iTunes gift certificate. They will not be cash money. The total value of incentives for attention retraining activities for any one patient for the entire study will not exceed $50. These incentives are not intended to be "compensation". Incentives are "reinforcements," a standard part of CBT. In CBT, as the burden of a therapy task increases, "tangible" reinforcements become more important.

Another phase of the cognitive training research will occur only among patients receiving treatment while not participating in the clinical trial of ABMT. This group of patients will undergo cognitive training for four weeks, during which time they also will be evaluated clinically by the research group and provided either CBT or fluoxetine treatment, based on clinical needs of the patient. In the main clinical trial of ABMT, an established form of cognitive training is used. However, unlike this established form of cognitive training used in the clinical trial, for this other phase of the cognitive training research, subjects will be studied with a newer form of training. This training has been studied in a few stand-alone trials of adult mood and anxiety disorders[165, 166] and in one initial trial of pediatric anxiety disorders[167]. This early-phase research suggests that the new form of training is enjoyable and potentially clinically helpful to patients with anxiety disorders. In the current protocol, subjects not participating in the ABMT clinical trial will participate in a feasibility phase of research, using the active form of this new form of cognitive training.

Of note, this new training uses a minor adaptation of procedures already used in the current protocol. Namely, this training uses the Dwell Time Eye Tracking task from the current protocol (see page 27, section 7.5.7.2.18). However, unlike the form of the paradigm described on page 27, when completing the therapeutic version of this task, subjects listen to enjoyable music that they select prior to initiating the training. Operation of the computer playing of the music is linked to the data for the eye-tracking patterns for the patient. With the cognitive training, playing of the music is suspended whenever the participant fixates gaze on a negative-valence face. Prior studies suggest that this alters gaze patterns in ways that reduces mood and anxiety symptoms[165, 167, 168]. However, for the current protocol, this aspect of the training serves only as an assessment of tolerability and feasibility.

7.5.3.2. *Weekly visits:* Children/adolescents and parents are seen as shown below by psychiatrists or psychologists for 30-40 minutes. Longer visits occur at weeks 0 and 8. A list of forms to be completed at each visit appears below.

Form	Informant	Time (min)	Inter-Viewer	ScreenWk -3	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8
KSADS	C, P	120	Therap	X								X
Neuropsych Testing	C	240	RA	X								X
Conditioning	--	45	RA	X								X
MRI Assessment	--	60-90	--	X								X
FHE/SCID	P	30	Therap	X								
Demographics	P	20	RA	X								
SCARED [83]	C	10	RA	X	X				X			X
CGI-S [84, 85]	C	5	MD	X	X	X	X	X	X	X	X	X
CGI-I [84, 85]	C	5	MD		X	X	X	X	X	X	X	X
PARS[86]	P, C	10	MD	X	X	X	X	X	X	X	X	X
ARI[145]	P, C	5	Therap	X	X							X
CBCL[87]	P	20	Self	X								

CDRS-R[87]	C	10	MD	X	X	X	X	X	X	X	X	X
CGAS[88]	P, C	5	MD	X	X				X			X
HARS[89]	P, C	5	MD	X	X				X			X
CDI[87]/MFQ	P, C	5	MD	X	X				X			X
Adverse Events[87]	MD	5	MD		X	X	X	X	X	X	X	X

KEY FOR ABBREVIATIONS IN TABLE APPEARING ABOVE: "KSADS" full= Complete Kiddie SADS; partial=anxiety and depressive modules of K-SADS; "FHE"=Family History Evaluation; "Demographics"=Demographic Interview; "SCARED"=Self-Report for Childhood Anxiety Related Disorders; "CGI"=Clinical Global Impressions (Severity and Improvement); "PARS"=Pediatric Anxiety Rating Scale; "ARI" = Affective Reactivity Index; "CBCL"=Children's Behavior Checklist Internalizing Scale; "CDRS-R"=Children's Depression Rating Scale-Revised; "CGAS"=Children's Global Assessment Scale; "HARS"=Hamilton Anxiety Rating Scale; "Adverse Events"=Fluoxetine Side Effects Scale; "Therap"=trained non-medical researcher or social worker, RA=research assistant, MD=child psychiatrist or pediatrician; P=parent; C=child.

7.5.3.3. *Assessments:* As shown above, adverse events are rated weekly. The primary efficacy measure comprises the CGI-Improvement rating, as this measure detected a significant effect of SSRIs on mood and anxiety disorders in both Emslie et al.[36] and the RUPP Anxiety Group Trial. Clinician ratings of anxiety and depressive symptoms are also completed weekly. Anxiety is rated with the Psychiatric Anxiety Rating Scale (PARS),[86] which detected a strong treatment effect of an SSRI on anxiety in the RUPP Trial. Depression is rated on the Children's Depression Rating Scale (CDRS), which detected a strong treatment effect of fluoxetine in Emslie et.al.[36] The Affective Reactivity Index (ARI)[145] will also be completed to assess clinican-, parent-, and child-rated irritability. The CGI Severity index is also completed weekly;[84, 85] it also detected a strong treatment effect in both studies. Other measures are assessed, beyond these main outcome measures, to detect clinical efficacy. These are assessed at randomization, week-4, and termination; they include: i) three self-rated scales, the SCARED,[83] MASQ,[90] and CDI,[87] and/or MFQ ii) the HARS and HAM-D;[89] and iii) the CGAS.[88]

7.5.3.4. *Dosage for Medication:* A flexible titration schedule is used, such that 10 mg of fluoxetine is given in week-1, followed by 20 mg from weeks 2-4; the dose can be raised by 10 mg in week five and 10 mg in week six for children who remain symptomatic. In addition, dose can be lowered for children who develop side effects.

7.5.4. Follow-up/Termination Procedures:

All patients are expected to complete all four phases of this study within six months, though precise timing depends on the needs of the families. Healthy volunteers are expected to participate over similar time frame, though healthy subjects do not receive treatment. Subjects will be terminated from the protocol when they request to discontinue participation. Moreover, subjects requiring clinical care after the completion of treatment at NIH will transition to community-based clinicians. In this instance, subjects may continue to participate by completing evaluations. However, these evaluations will only be performed for the purposes of research. Clinical care will be provided in the community, outside of the current protocol.

Research-based follow-up evaluations will be performed on all subjects. Return single day visits will be scheduled no more frequently than every 6 months after completion of treatment of the last visit. These visits will extend for up to two years. At these follow-up evaluations, interim psychiatric histories, clinical measures, and diagnostic interviews may be repeated. For subjects who are willing, additional MRI exams and EMA assessments will be completed, as already specified in the protocol. In addition, consent form signatures will be obtained on a yearly basis. Phone interviews will be conducted if participants are unable to come in for a visit. To minimize the length of the visit, self-report measures will be mailed to participants, filled out at home, and returned to the investigator. The remaining procedures will be completed during one visit. Of note, subjects will not be eligible to receive treatment regardless of the outcome of these visits. However, the study team will work with families to place patients with appropriate outside clinical services should the need for such placement arise.

Subjects will be assessed using a psychiatric history, clinician ratings, and standardized measures. Subjects who are evaluated at follow-up will not be eligible to re-enroll in the treatment phase. If a subject meets diagnostic criteria for a disorder meriting psychiatric attention, the clinical staff will work with the family to obtain the appropriate treatment in the community.

7.5.4.1. *Psychiatric History:* Subjects may be screened for psychiatric disorders using the K-SADS Interview,[63] administered by a trained clinician (at least Masters level) supervised by Dr. Pine. This measure requires one-to-two hours to complete. In adults, the SCID Interview will be used in place of the K-SADS. All adult subjects will be screened for psychiatric disorders using the SCID Interview.

7.5.4.2. *Child Ratings*: Adverse events will be rated each year. Self-report and clinician ratings assessed weekly during the treatment phase will be repeated every year. New measures will also be added to assess other domains of functioning. These measures include the Friendship Questionnaire (FQ), Self-Perception Profile for Children and Adolescents (SPPC/SPPA), the Network of Relationships Inventory (NRI), and the Social Skills Rating System (SSRS).

7.5.4.3. *Adult Ratings*: Adult ratings may consist of the Compiled Mood & Anxiety Rating Scales (C-MARS) which includes the MADRS, SIGH-SAD, IDS, YMRS, and the HAM-A. Adults will also complete the Beck Depression Inventory II (BDI-II), the Beck Anxiety Inventory (BAI), the Sheehan Disability Scale (SDS), the Inventory of Interpersonal Problems (IIP), and the Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q).

8. Risks/Discomforts:

8.1. Psychiatric Assessment:

Based on our research and clinical experience with children and adults to date, no adverse reactions are anticipated as a result of the assessment procedures. For most of our studies, the task of completing the face-to-face interviews, tests, and scales will require a maximum of five hours for both parents and children. It is highly unlikely that a study participant will become upset about the questions or interview process used in the study. Each measure has been used extensively in hundreds of children without adverse effects. Children may refuse study participation at any time, and the standard interviewing and data collection procedures will cease, should any adverse reactions be noted. All subjects completing the interviews and questionnaires will be provided with phone numbers so that they can have any questions answered that they feel have not been satisfactorily addressed. The investigative team has extensive interview experience, and they will determine if there is a need for clinical intervention; if necessary, arrangements for appropriate clinical services will be made. Data for ecological momentary assessments (EMA) and the DAWBA will be collected using secure technology, with procedures approved by the NIMH ISSO.

The plan for obtaining DAWBA data are as follows: After consent, our team will provide the patient and/or his or her parent or legal guardian each with a unique username and password, either in person, over the phone, or via medical secure email (MSE), that will allow them to log onto the website. Only our NIH lab team and no other party will have access to the file key that links the username and password to the patient name; this file will be stored on our secure NIH server and only accessible to our NIH staff. The patient and/or parent or legal guardian can then log onto the secure website and complete the interview. At no point is the patient and their parent or gaurdian asked for any personally identifiable information (PII) as defined by the NIH or HIPAA. Within the instructions that given to the patient and parent or guardian and at the introduction of the online interview, it is explicitly stated that no PII is required. Our lab team will download the interview data within one week from its completion on our secure server and once this has been

accomplished, the data are permanently deleted from the online server. In addition, any DAWBA forms that have not been downloaded to our server, and thereby removed, will be purged from the web application within two months of their completion.

8.2. Neuropsychological Indices:

Procedures for neuropsychological tests as well as neuropsychological aspects of the fMRI tasks are also considered minimal risk. There are no more than minimal risks associated with memory and attention tasks, as the tasks are very similar to standard neuropsychological measures and involve experiences that children undergo on a regular basis. While tasks do present faces making scared expressions, such expressions are encountered regularly by children in their daily lives, in books, magazines, and movies. Similarly, mildly upsetting pictures will be presented from the International Affective Picture System (IAPS) slide series, but these pictures are no more upsetting than those that are routinely presented in prime-time media. The IAPS stimuli presented will be from appropriate subsets of the IAPS 'catalogs,' choosing only from catalogs of animals and insects, human subjects, nature, familiar objects, food, sports and recreation. All catalogs, and thus all individual pictures, with sexual content or themes of violence, death, or eroticism have been eliminated from consideration. Parents of all minor subjects will have an opportunity to review and approve all IAPS pictures and pictures depicting harmful acts. Subjects will be exposed to loud noises, air-puffs, and thermal heat as part of the fear-conditioning paradigm. However, this degree of loudness and this level of air-puff has been used in many prior studies, and adolescents regularly are exposed to stimuli of similar intensity. Subjects will have the opportunity to win or lose money during the participation of reward tasks. However, it will not be possible for subjects to lose any money, beyond the money that they first earn as part of participation. Subjects will participate in mildly stressing tasks (e.g., unsolvable anagram task, social dyad task, social stress task) as a means to test stress-reactivity following attention retraining procedures. These tasks mimic real-life stressors that the subjects encounter and are limited to 5-10 minutes in duration. Finally, subjects are exposed to paradigms involving deception. Subjects are notified of this possibility at consent, and subjects are debriefed following study participation.

8.3 CO_2 Challenge Task: The main risk of the CO_2 procedure is that children may become anxious. Dr. Pine and his colleagues have conducted more than 400 CO_2 exposures using a 5% CO_2 concentration gas mixture. To minimize risk, only children who are at least 9 years old will be studied with this procedure for the current protocol. Dr. Pine's experience includes completion of many such challenge procedures, conducted in the NIH Clinical Center, performed in collaboration with Dr. Roberson-Nay. These exposures occurred in children between the ages of nine and 18. These include more than 75 children with no history of mental disorders and more than 75 children with a current anxiety disorder. Dr. Roberson-Nay has continued to adapt the CO_2 challenge procedure for use with children at the Virginia Commonwealth University. She has found that the 7.5% procedure produces more robust results than the 5% procedure, having performed more than 100 such studies in subjects of various ages. Of most relevance to the current protocol, in the past six months, she has conducted 20 7.5% CO_2 challenge procedures in children between the ages of 9 and 11, the youngest group to be included in the current protocol. In these 20 children, the mean ***peak*** anxiety rating during inhalation of CO_2 was 3.4 (SD=2.1) on a 0-8 point scale. Peak anxiety ranged from 0 to 6, with the modal anxiety rating being 2. Thus, inhalation of 7.5% CO_2-enriched air is generally considered mildly anxiety-provoking in most children ages 9-11. Moreover, this study in 9-11 year-olds included a reliability session. Eighteen of 20 participants returned to participate in the CO_2 challenge a second time. Because concerns about the exact consequences of becoming anxious are likely to occur, we describe at some length the evidence bearing on this procedure. Much of the relevant work has been done with adults. Of note, Dr. Roberson-Nay received extramural support from the NIH to implement this work, where this procedure was deemed to be minimal risk by the relevant NIH review group.

CO_2 Exposure in Adults: Questions on the potential for adverse effects following anxiety-provoking procedures have led to considerable systematic research on the potential for such effects following CO_2 exposure. The research has failed to reveal any evidence of adverse effects.[106-109] Harrington et al.[106] assessed 62 adults in the year after 37.5% CO_2 exposure, a procedure which induces significantly greater anxiety than the 7.5% CO_2 inhalation procedure to be used in the proposed study. No evidence of adverse effects was found. Similar conclusions were reached by Perna et al.,[109] who followed 34 adults over a three-to-four year period after they underwent 37.5% CO_2 inhalation. Again, no evidence of adverse effects was found. Three aspects of the work from Perna et al.[109] are particularly relevant for the current proposal. First, Perna et al. have re-assessed over the past three years a larger proportion of their sample studied with the CO_2 inhalation procedure, again finding no evidence of adverse effects. In total, Perna's group has conducted over 750 37.5% CO_2 inhalation procedures and has found no evidence whatsoever of adverse effects (G. Perna, personal communication, October 1998). No report has occurred of an exacerbation of anxiety following the procedure. No single new-onset anxiety disorder has resulted from the procedure. Second, Perna et al.[109,110] assessed the psychiatric sequelae of CO_2 inhalation in first-degree relatives of patients with panic disorder, and found no evidence of adverse effects in this high risk group. Third, the studies of Perna et al.[109,110] include children between the ages of 15 and 18; they also failed to detect any evidence of adverse effects in this age group. In summary, as with Harrington et al.,[106] data from Perna et al.[109,110] indicate no risk for adverse effects in individuals who experience CO_2-induced anxiety.

Finally, data from two other groups are consistent with the experiences of Harrington et al.[106] and Perna et al.[109,110] in suggesting that adverse effects do not follow CO_2 exposure. Members of our research team have systematically re-evaluated more than 100 adults in the period after respiratory provocation tests, again uncovering no evidence of adverse effects. Similarly, the Iowa research group (W. Coryell, personal communication, October 1998) has studied more than 120 adults with the CO_2 inhalation procedure, approximately half of who were first-degree relatives of patients with panic disorder. Again, no evidence of adverse effects was noted, confirming the observations from Perna et al.[109,110] Moreover, Coryell has conducted repeated CO_2 inhalations in a sizable portion of this sample, including relatives of patients with panic disorder, again noting no evidence of adverse effects, either clinically or biologically, in the response to repeated CO_2 exposure. In fact, data from Coryell and others on the response to repeat CO_2 inhalations are far more consistent with habituation than with sensitization effects. The response to multiple CO_2 inhalations in anxious adults consistently decreases with repeated exposure, leading some experts to argue that the procedure has therapeutic, rather than adverse effects, through desensitization.[106-108] In summary, available data reveal no evidence of adverse effects in adults, and are more consistent with habituation than with sensitization effects. The studies described above show that adverse effects do not follow CO_2-induced anxiety among adults.

CO_2 Exposure in Children: The most persuasive evidence that exposure to CO_2 presents minimal risks to children is our extensive prior experience. In our studies with more than 400 children, we have had no complaint from any child or parent that the procedure was experienced as anything more than transiently anxiety-provoking. In all children, the increase in anxiety was transient, lasting on the order of minutes. All children were completely free of anxiety, typically within 30 minutes of the CO_2 procedure.

The assessment of respiratory physiology in this age group, including CO_2 inhalation, is considered a minimal risk procedure. This conclusion was reached by Institutional Review Board considerations at five institutions, including the National Institute of Health Intramural Research Program and four other institutions (Long Island Jewish Medical Center, New York State Psychiatric Institute, Columbia University College of Physicians and Surgeons, and Virginia Commonwealth University) where the research team has conducted CO_2 inhalation studies. There is no known medical risk associated with the procedures. Our group has conducted more than 400 CO_2 inhalation procedures in children between age seven and 18, without any adverse outcomes. Children with anxiety disorders do run the risk of becoming transiently anxious while inhaling CO_2, and this risk is highest before the age of nine. However, as described above and

below, the degree of anxiety is mild-to-moderate and short-lived. In this study, CO_2 will be delivered through a face mask that children will hold up to their face. In the event that the child becomes anxious, the child can stop the delivery of CO_2 by simply removing this mask from his or her face. This is explained and demonstrated to each child. Children who have become anxious in our prior studies have all been asymptomatic within minutes of the procedure. As also outlined below, follow-up contact with these children confirms that any degree of discomfort is transient.

To evaluate the potential for adverse effects in children in our past work, we conducted four types of assessments. i) We re-contacted a consecutive series of 85 children in the weeks after the CO_2 inhalation procedure. There was no report, in any one case, of worsening anxiety following the procedure. We followed children in two studies as they received serial CO_2 inhalations. In one of these studies, children participated in four inhalation procedures, over a 10-day period. In the other, children participated in inhalation procedures before and after being treated for an anxiety disorder over a two-month period. Children in this latter study were systematically followed for the year subsequent to the final CO_2 inhalation procedure. A total of 35 children were examined in the two studies. As found among adults, children's level of anxiety decreases across repeated procedures, findings that are more consistent with habituation than with sensitization effects. In no case did a child exhibit more anxiety in subsequent procedures. Moreover, there was no other evidence of any adverse effects from the procedure. Finally, to re-evaluate children over longer periods, we re-contacted a consecutive series of 100 children who participated in a CO_2 inhalation study more than 10 years previously. Again, we uncovered no evidence of adverse effects in these assessments.

In summary, a multitude of observations in children and adults find that CO_2-induced adverse effects do not occur. CO_2 inhalation does not precipitate clinical anxiety disorders in children or adults who are free of an anxiety disorder. CO_2 inhalation does not exacerbate the clinical condition of children or adults with an ongoing anxiety disorder.

Our team will take many steps to increase the tolerability of the procedure. These steps might contribute to the generally positive experience reported by most families in our prior work, even when the procedure produces anxiety. Before the procedure, each family and child meets with a physician for approximately 15 minutes, during which time the relationship between anxiety and respiration is discussed. Families learn about our prior studies on CO_2 sensitivity in children and adults. They are taught about theories, which attribute anxiety and panic to respiratory dysfunction. At this meeting, the physician explains that children should ask to terminate the procedure at any point they begin to feel anxious and that the integrity of the study depends upon children being very forthcoming in reporting any feelings of anxiety they develop. In the event that a child does become anxious during CO_2 exposure, the physician provides reassurance by letting the child know that any discomfort the child is experiencing will dissipate within minutes and will help the child engage in slow diaphragmatic breathing. Children are asked if they would like to sit with their parent, if the parent had not been with the child during the procedure, and the child is given water or juice, if so desired. The child is then asked to review the symptoms that were experienced during the exposure, with reference to the usual symptoms of a panic attack. The physician then explains that CO_2 might be capable of provoking anxiety in the child due to an increased sensitivity of the subject's nervous system. In all cases, children have been reassured by this explanation and have been without any anxiety by this point in the procedure.

8.3. Functional MRI (fMRI):

Functional brain uses MRI to observe activity-related, hemodynamic changes with high spatial and temporal resolution. The most promising method, as is used in the current protocol, examines changes in blood oxygenation. Because of the different magnetic properties of oxy- and deoxyhemoglobin, increased levels of blood oxygenation are associated with increased signal for some MR imaging methods, including T2*-weighted gradient echo imaging and echo-planar imaging. Increased neural activity is associated with this increased signal, presumably because local, activity-related increases in blood flow are greater than increases

in oxygen extraction, resulting in higher ratios of oxy- to deoxyhemoglobin. Because the change in signal is due to properties inherent in normal blood constituents, injection of contrast agents or tracers and sampling of plasma are unnecessary.

All studies in the current protocol involve presentation of visual stimuli while the subject is in the scanner, and the subject responds by pressing buttons with the thumb of his dominant hand. Most studies will be performed using a GE 3 Tesla scanner. A smaller set of studies will be performed using the Siemens 7 T Terra using the Nova 1Tx/32Rx head coil. At 7 T, only subjects who weigh more 66 pounds will be eligible, based on safety considerations.

All scanners are located in the In Vivo NMR Center in the Clinical Center. During a scan the subject hears a loud rhythmic tapping or banging sound. This noise is caused by the switching of the gradient coils that is necessary to produce the image. Subjects are warned of this prior to scanning, and most do not find it to be objectionable. Protective earphones and earplugs are provided.

MRI is widely regarded as a safe, noninvasive procedure for visualization of brain tissue in both adults and children. The risks involved with fMRI or DTI are the same as those involved in standard anatomic MRI, since these procedures rely on the same physical properties of brain tissue. As noted above, most procedures for this study will be performed on an FDA approved 3T scanner at the NIMH. At the time that this protocol began in 2001, FDA standards for minimal risk MRI required that four criteria be met: 1) a static magnetic field no greater than 4T; 2) specific absorption rates a) no greater than 4W/kg for the entire body for 15 minutes, b) no greater than 3W/kg over the head for 10 minutes, and c) no greater than 8W/kg in any gram of tissue in the head or torso or 12W/kg in any gram of tissue in the extremities for five minutes; 3) a time rate of change in the field that does not produce physical discomfort or painful nerve stimulation; and 4) a peak sound pressure level that does not exceed 140 dB or A-weighted R.M.S. pressure level that does not exceed 99dBA with hearing protection. Each of these guidelines will be monitored throughout the study to ensure that none are exceeded.

FDA standards for minimal risk MRI required that four criteria be met: 1) a static magnetic field no greater than 8T; 2) specific absorption rates a) no greater than 4W/kg for the entire body for 15 minutes, b) no greater than 3.2 W/kg over the head for 10 minutes, and c) no greater than 8W/kg in any gram of tissue in the head or torso for 15 minutes or 12W/kg in any gram of tissue in the extremities 5 five minutes; 3) a time rate of change in the field that does not produce physical discomfort or painful nerve stimulation; and 4) a peak sound pressure level that does not exceed 140 dB or A-weighted root mean square (rms) sound pressure level greater than 99 dBA with hearing protection in place.

MRI at 3 Tesla is used on occasion for clinical scanning but is widely used for research. This includes studies with healthy children. The gradients for the GE 3 T scanner are higher performance, in terms of gradient strength and slew rate, with a maximum gradient strength of 4 G/cm. No studies have documented any detrimental effects of gradient magnitude. The maximum slew rate, the maximum rate at which the gradients change in magnitude, of the gradients on the 3T scanner is 150 mT/m/sec. A higher maximum slew rate allows for faster imaging which is generally better because the shorter time to collect an image causes images to be less contaminated by susceptibility or motion artifacts. It is well known that if the gradients are switched too rapidly, peripheral nerve stimulation can take place.[91] The 3T scanner has standard safeguards such as a maximal allowable slew rate that is 66% of the FDA limit. Lastly, because of the higher radio frequencies associated with the 3 T scanner, larger amounts of energy are deposited into the tissue during scanning at 3 T than at lower field strengths. Tissue heating becomes a concern for certain pulse sequences at higher field strength. Again, the operating system for the scanner has built-in safeguards that only allow

scanning with specific absorption rates (SAR) of radio frequency that are well below guidelines established by the Center for Devices and Radiological Health (CDRH), FDA. The operating system limits radio frequency deposition in the head to an average rate of 10 watts, < 3.2 w/kg over 10 minutes, which has been shown to raise the average core temperature approximately 0.3 deg. C.[92] These temperature changes are within the normal diurnal rhythms (±1°C) found in human core temperatures or a change associated with a brisk 20 minute walk (+1°C).

As noted above, some evaluations in the current protocol will be performed at 7 T. Since 2003, the FDA has considered MRI studies done at 8 T or less (i.e. 7 T) to pose no significant risk. The guidance specified that RF energy absorption, noise, and peripheral nerves stimulation are the same for 7 T and 3 T. This guidance was for the general population – (infants more than 1-month-old, children and adults). In late 2010 scientists at the NIH began scanning routinely at 7T , and more than 5000 exams at 7 T have since been performed at the NIH. This has generated had an excellent patient safety record at 7 T. In 2017 the FDA gave 510(k) clearance to Siemens for the 7 T Terra using the Nova 1Tx/32Rx head coil using FDA-cleared pulse sequences. These are the devices that we will use with our subjects. Siemens requested and received clearance for subjects weighing more than 66 lbs (30kg). This provides the rationale for limiting subjects who weigh at least 66 lbs.

Some of the MRI sequences used in this study do require scanners be run in "Research Mode" using research sequences. However, of note, this is always performed in a fashion termed the "First Controlled Operating level". As such, whenever research sequences are run, the First Controlled Operating level is the level under which the MRI scanner is running. With this level, all scans performed by the scanners remain within the FDA's safe operational limits for gradients, dB/dt recommendations, and radio-frequency (RF) performance. As a result, the FDA considers this mode of operation for our scanners to be NSR. Given this NSR designation and the levels for gradients and RF performance, no additional risk is added beyond the typical "Clinical Mode" under which the scanner operates in the community. Finally, FDA approved hardware safety measures are operational during scanning so that FDA guidelines are never surpassed. With these measures, procedures are put in place in the scanner to make sure that these safe operational limits cannot be exceeded and to make sure that all scans remain NSR. These procedures are part of scanner operations in the First Controlled Operating level and additional levels involve additional passwords and keys that are not enabled when we acquire data

MRI has not been approved by the FDA for the research techniques used in this study. We will operate the MRI scanner in a research mode to test and improve the pictures. Specifically, we will use research pulse sequences and image reconstruction and analysis software in the MRI study. Some scans will also use a non research, FDA approved coil: Nova Medical 3T 32 Channel Head Coil (U.S. FDA 510(k) Clearance: K182737). The Sponsor has determined that the device is used in the trial in accordance with this label. There is no known increase of risk in the use of these research scan techniques compared with those used in clinical scans.

The current techniques are considered NSR because they do not meet any of the criteria for a SR device. Under 21 CFR 812.3(m), an SR device means an investigational device that:

- Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject;
- Is purported or represented to be for use supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject;

- Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject; or
- Otherwise presents a potential for serious risk to the health, safety, or welfare of a subject.

Some people may experience physical discomfort during scanning, including brief periods of muscle twitching, eye discomfort, dizziness, mild nausea, headache, or a sensation of flashing lights. We will make sure that every participant feels comfortable to let us know if they feel any discomfort, and most importantly, to let us know if they want to stop the study. We will stop the study as soon as a participant communicates to us that he/she wants to stop the study.

Unless they decline, each child who undergoes fMRI in our laboratory trains in a simulator device to increase familiarity and reduce any stress associated with the novelty of the procedure. We also interview each subject who completes our fMRI studies following the study. Children rate a set of negative emotions (e.g. "scared", "angry", "grouchy") using 10-point scales, with a score of 1 indicating "no" degree of negative emotion and a score of 10 indicating extreme degrees of negative emotion.

8.4. Magnetoencephalography (MEG)/Electroencephalography (EEG):

There are no known risks associated with either MEG or EEG. Both are completely non-invasive and are routinely recorded in infants, children, and adults in laboratories across the world without incident. However, it is possible that some children may feel minimal discomfort due to the EEG net placed on their scalp. We will make sure that every participant feels comfortable to let us know if they feel any discomfort, and most importantly, to let us know if they want to stop the study. We will stop the study as soon as a participant communicates to us that he/she wants to stop the study.

8.5. Treatment:

Fluoxetine is used in the current protocol in an IND-exempt manner. The justification for IND-exempt use reflects the facts that the medication is lawfully marketed in the United States, the study is not intended to support a new indication or any other significant change in product labelling or advertising, and the fact that the medication is widely used to treat a range of pediatric mental health conditions in the community. In light of the high frequency of use in the community for the conditions studied in the current protocol, the study cannot increase risks as compared to treatment in the community. In fact, throughout the community, most uses of selective serotonin reuptake inhibitors represent uses for conditions where there is no FDA indication. Specifically, fluoxetine and other selective serotonin reuptake inhibitors exhibit larger clinical effects with equal tolerability in the treatment of anxiety disorders, as compared to major depressive disorder and obsessive compulsive disorder, the two pediatric conditions for which fluoxetine has an FDA Indication (see Locher et al. 2017).

In adults, fluoxetine's side effects can include: nausea, constipation, loss of appetite, diarrhea, indigestion, sleepiness, fatigue, mouth dryness, insomnia, headache, nervousness, dizziness, tremors, increased sweating, irritability and anxiety. In prior studies among children and adolescents, these symptoms have been mild, to the point where fewer than 20% of subjects require dose adjustment. Altered appetite and weight can occur in approximately 10% of adult patients. Weight changes will be monitored; if the weight falls to a clinically-significant degree, medication will be discontinued. As with most antidepressants, mania may occur with fluoxetine, as reported in approximately 1% of patients. Some patients have been reported to have an increase in suicidal thoughts and/or violent behavior with fluoxetine. Initial concerns in this area led the

Food and Drug Administration to undertake a systematic review of the data on suicidal thoughts during SSRI treatment in youth and adults. The results from this inquiry led to placement of a Boxed warning on all antidepressants specifically for individuals age 25 or younger. This warning notified the public about the risk for suicidal thoughts and aggressive behaviors with all antidepressants, including fluoxetine.

Finally, subjects with anxiety disorders will be exposed to a novel computer-based attention-retraining task or a control task, with similar procedures to the retraining task. These tasks involve repeated exposures to photographs of faces. As the procedures involved in these tasks are similar to those encountered by children interacting with many forms of media, no adverse events are anticipated. However, this task will train subject to shift their attention away from angry faces. There is some concern for children who do not possess an attention bias towards threat that this procedure could interfere with CBT or fluoxetine treatment.

9. Subject Monitoring

9.1 Procedures for Monitoring*:*

As noted above, the current study involves one of two treatments, either a) eight weeks of open treatment with fluoxetine for children/youth and adults or b) Open Cognitive Behavioral Therapy with youth during which time clinical response will be monitored weekly by a psychiatrist or psychologist. Families will be provided with phone numbers for emergency coverage, 24-hours/day. Clinical response will be rated on the CGI-I Scale. This scale incorporates all clinically relevant data that is gathered during the trial. This includes data from the subject, parents, or other sources, as well as data from the clinician's observations. As a result, the assessment of clinical response, as rated on this scale, will comprehensively capture the global clinical state of the subject and the need for intervention, should patients begin to deteriorate. In addition, adverse events associated with medication will also be monitored on a weekly basis.

9.2. Procedures for Removing Subjects from Trial:

Subjects will be removed from the study by the research team under three circumstances. First, in each instance where a subject shows clinical worsening or clinically significant adverse events, details of the case will be reviewed independently by three clinicians during the first visit where the subject shows worsening as well as the next visit. Results of this review will be discussed with the patient and families. Clinical worsening is defined as a rating of "7" ("worse") or greater on the CGI-I for any visit. Subjects will be removed from the study if they are rated as "worse" by two of the three clinicians for two consecutive weeks, though families are free to discontinue the trial at any point. Second, subjects will be removed if they exhibit clinically significant increase in suicidal ideation.

10. Outcome Measures:

The main outcome measures, related to the primary study goals, derive from the fMRI paradigms. These measures represent indices of percent-signal change in hypothesized brain regions, comprising amygdala, striatum, and prefrontal cortex (PFC). In each fMRI paradigm for each of these brain regions, a measure of percent-signal change is generated for the main study contrast for each paradigm. These percent-signal change measures are also used as predictors in analyses related to secondary study goals. Similar neural activity data will be obtained using MEG.

One set of key secondary outcome measures derives from the treatment-response data. Treatment response is defined based on one categorical measure and one continuous measure. The categorical measure is the Clinical Global Improvement (CGI) score, and the continuous measure is the Pediatric Anxiety Rating Scale

score (PARS). Other assessment measures collected during treatment (e.g., parent or child ratings on symptom scales) serve to augment the main analysis, based on the CGI and the PARS.

A second set of key secondary outcome measures derive from the pre-assessment clinical and information-processing assessments. Patient groups will be compared with each other and with healthy subjects in analyses that are designed to clarify factors that contribute to findings for the fMRI and treatment-related data. Thus, clinical groups will be compared based on their symptom profiles and their performance on information-processing tasks. In addition, physiological data also will be compared at baseline. This includes measures of fear conditioning and response to CO2. To the extent that findings emerge in these analyses, symptom profiles and information processing tasks also will be used in analyses of fMRI and treatment-outcome data.

11. Statistical Analysis:

11.1. Processing of fMRI and M/EEG Data: Steps will be implemented to prepare neuroimaging data for analysis.

11.1.1. *Maintenance:* Data will be offloaded from the scanner to workstations immediately after the scans have been acquired. These data will then be archived and stored at NIH.

11.1.2. *fMRI Data Processing*: Analysis involves a series of initial steps including realignment (i.e., correction for subtle head movement), stereotactic normalization, resampling and spatial smoothing. Data from any subject with movement greater than 4 mm in any direction will be discarded. Stereotactic normalization, re-sampling, and spatial smoothing will use standard methods. Results of functional image analyses are co-registered for presentation of group results. Of note, analysis of M/EEG data will follow similar principles.

11.1.3. *Regions-of-Interest (ROI) Analyses*: We will use both a voxel-wise and region-of-interest (ROI) approach to our data. ROI analyses will be used to test a priori hypotheses, while a voxel-wise approach will be used in exploratory analyses. ROI analyses are possible due to a priori hypotheses on regional differences in activation profiles. Identification of ROI's will use established methods for cortical and subcortical parcellation of frontal (superior, inferior, orbital, and anterior cingulate) and temporo-limbic (superior temporal gyrus, amygdala, hippocampus) regions on high resolution T1 weighted images.[93, 94] ROI's will be selected based on hypothesized areas of activation in each task. In general, ROIs will include the amygdala and inferior/orbital prefrontal cortex.

11.2. Data Analysis for fMRI and M/EEG Data:

11.2.1. *Statistical Methods for Assessing Task-Associated Activation in All Groups*:

11.2.1.1. Procedures*:* Hypotheses on task-associated changes in BOLD signal will be tested by analyzing task effects on CNS hemodynamic responses. Two sets of analyses will be implemented. First, to test a priori hypotheses on regional task-associated changes, we will use random regression procedures similar to those outlined above in the analysis of clinical data. However, for imaging analyses, average BOLD signal in a specific ROI will serve as the dependent measure, and across-time changes in BOLD signal during the experiment will be modeled. Second, to conduct exploratory analyses of other regional differences in task-associated change in BOLD signal, we will use SPM. For both sets of analyses, procedures will draw heavily on the expertise of Eric Zarahn, Ph.D. in our group to model effects on BOLD activity of specific events occurring within larger blocks of events on BOLD activity. For example, we will model the effects of specific emotion faces on BOLD activity when these faces are presented as part of a larger series of faces. Full details of these methods are provided elsewhere.[75-78] These voxel-wise analyses of spatially smoothed contrast images will rely upon random effects models, as previously implemented by

Zarahn and colleagues.[75-78] Analyses of M/EEG data do not rely on the CNS hemodynamic response; apriori analyses of these data will involve identifying specific sensors and time and/or frequency windows of interest, during which the M/EEG measures will be contrasted across task conditions. Additionally, exploratory analyses of M/EEG data may involve using cluster permutation tests to empirically identify groups of sensors, time points, and frequencies that significantly differ across task conditions.

11.2.1.2. Statistical Power***:*** Power computations focus on tests of a priori hypotheses implemented through ROI analyses of fMRI data. These computations use conventional statistical approaches, where the average BOLD signal intensities in a given ROI are treated as the dependent variable. In general, with sample sizes as small as n=15, alpha = .05, two-tailed, we would have power of >.80 to detect effect sizes of 1.2 standard deviation activations. Most fMRI studies of neurobiologically meaningful task-associated changes in BOLD signal document larger effects than this. As noted above, we will also conduct exploratory analyses using a voxel-wise approach to consider changes in task-associated BOLD signal within other regions. These considerations apply to each of our neuroimaging tasks.

11.2.2. *Between-Group Contrasts:*

11.2.2.1. Statistical Methods***:*** To test a priori hypotheses in each cognitive task, contrasts will first model task-associated changes in BOLD signal in specific ROIs as a function of three factors: (a) clinical group (healthy vs. impaired), (b) stimulus characteristics (e.g. nature of faces in vigilance paradigm; valence of pictures in memory paradigm), (c) task-related performance variables (reaction time in vigilance paradigm, stimulus ratings or memory performance in memory paradigm). In secondary phase, specificity of associations across individual diagnoses will be examined (e.g. major depression). Following these analyses, contrasts next will model between-group changes for impaired subjects in BOLD signal before and after treatment by including a treatment factor and repeated measures design in these models. Finally, we will use a voxel-wise approach to conduct exploratory analyses designed to model between-group differences in regions beyond those considered in the ROI analyses. Of note, an identical approach can be employed in studies of specific clinical conditions (e.g. anxiety vs. depression) as well as specific age groups (adults or youths). Finally, because only adolescents are studied before and after treatment, within-subject analyses on treatment effects will not be performed in adults, comparing fMRI data before and after treatment. However, adults may be studied on two occasions to generate measures of stability. As above, apriori analyses of the M/EEG data will involve identifying specific sensors and time and/or frequency windows of interest, during which the M/EEG measures will be contrasted across participant group. Additionally, exploratory analyses of M/EEG data may involve using cluster permutation tests to empirically identify groups of sensors, time points, and frequencies that significantly differ across participant group.

11.2.2.2. Attention Bias Paradigm***:*** In prior behavioral studies, enhanced attention bias has been found in both depression and anxiety, though more consistently in anxiety disorders. Enhanced attention bias occurs in both anxiety and depression when evocative stimuli are presented supraliminally but only occurs in anxiety when evocative stimuli are presented subliminally.[28] Because Mogg and Bradley[17, 27, 28]are the only group to demonstrate supra versus subliminal dissociations in attention bias using faces, only broad hypotheses can be generated for the current neuroimaging study. The initial set of analyses will test hypotheses on differential engagement of brain regions during trials where a cue consists of an angry "threat" face. The key hypothesis is that ventral fronto-limbic regions will be engaged by "angry/threat" as opposed to "neutral" faces. This effect is expected to differ between healthy and impaired youth; based on Mogg and Bradley,[17, 27, 28]the effect is hypothesized to occur only in anxiety when faces are presented subliminally but in both anxiety and depression when faces are presented supraliminally. This effect is also expected to correlate with the degree of vigilance, as reflected in reaction time advantages for "threat" faces. Treatment is hypothesized to normalize association between disorders and vigilance. As a result, the initial data analysis of a priori hypotheses will use a ROI-approach to model blood oxygen level dependent (BOLD) signal as a function of face trial characteristics ("angry/threat" vs. "neutral" vs. "happy"), threshold (supra vs. subliminal), reaction time/vigilance performance, and psychiatric status. These analyses will use

an event-related approach. Namely, by using stochastic methods, data analysis will decompose changes in BOLD signal as a function of processes related to each of these factors. Following treatment, a time factor (pre vs. post-treatment) will be added to these analyses. After these initial analyses, exploratory analyses will use a voxel-wise approach to consider other regional effects beyond those that are examined in the ROI analyses.

11.2.2.3. Emotional Memory Paradigm***:*** For the emotional memory paradigm, data analysis will contrast brain regions engaged during the viewing of faces, film clips, or pictorial stimuli. These analyses will map brain regions engaged by the paradigm as a function of stimulus content (e.g., negative or positive valence) and rating instructions (e.g., external features, valence of the stimulus, participant's anxiety). For faces, data analysis will model brain regions engaged by particular emotional expressions depicted in the faces. Similarly, for pictures, data analysis will model brain regions engaged by high-arousal/negative valence pictures. For film clips, analysis will model brain regions engaged based on the emotional expression in the depicted individual (e.g., positive versus negative valence). Thus, by using stochastic methods, data analysis will decompose processes both related to the nature of the rating process and related to nature of the stimulus. Impaired and healthy subjects are hypothesized to differ both in the degree to which limbic regions are engaged by the rating process and by their emotional memory for stimuli.

Using an ROI approach, the initial data analysis will model the independent relationships that emotional memory and psychiatric status show with brain activity. One key set of hypotheses suggest that ventral fronto-limbic regions (e.g. anterior cingulate, orbital frontal cortex) will be selectively engaged by rating emotional features as opposed to physical features of stimuli. This effect is anticipated to differ between healthy and impaired youth; treatment is hypothesized to normalize this effect. A second key set of hypotheses is that emotionally evocative pictures and faces will engage the amygdala, irrespective of rating instructions, particularly among anxious/depressed youth; treatment is also hypothesized to normalize this effect. The third set of key hypotheses suggest that amygdala activation at encoding will predict emotional mnemonic bias after scanning, while hippocampal activation at encoding will predict overall memory performance.

Based on these hypotheses, initial data analysis will model average blood oxygen level dependent (BOLD) signal in the relevant ROI as a function of trial characteristics (e.g., face expression, IAPS type), rating set (e.g., "valence" vs. "luminosity"), memory performance ("remembered" vs. "not remembered"), and psychiatric status. As with the vigilance data, these analyses will use an event-related approach. Namely, by using stochastic methods, data analysis will decompose processes related to each of these factors. Following treatment, a time factor (pre vs. post-treatment) will be added to these analyses. Finally, as with the analysis of data from the vigilance experiment, exploratory analyses will also be implemented in the memory experiment, using a voxel-wise approach. These analyses are designed to examine effects in brain regions beyond those specified in the ROI analyses.

11.2.2.4. Other Paradigms: The analytic approach for reward, social interaction, fear conditioning, and Affective Posner paradigms will be very similar to that used for the attention and memory paradigms.[65-68, 70] Thus, key events will be selected, based on hypothesized psychological processes implicated in anxiety or depression. Data analyses then will use the same event-related methods described for the attention and emotional-memory paradigms. The key events of interest for reward tasks represent the omission and delivery of expected rewards, for social interaction tasks represent "rejection" events, when subjects receive feedback informing them of the rejection, and for fear conditioning represent conditioned fear cues. These events will be contrasted with various control events, including baseline events, and events with neutral valence. The key regions to be examined comprise the amygdala, ventral prefrontal cortex, and striatum.

11.3. Clinical Data:

Relative to placebo attention training, active attention training is hypothesized to produce greater decreases

in anxiety symptoms and higher rates of response. To test this hypothesis for continuous outcome measures, we will contrast study groups, defined by attention-training conditions, using random effects regression (for continuous measures)[95] or using logistic regression (for dichotomously-defined CGI outcomes). Parallel logistic regression models will be used to predict treatment response, defined as a CGI rating that is better than "4" (4=minimally improved). This hypothesis is tested as the main effect of treatment group on CGI category (responder vs. non-responder). In all analysis, the intent-to-treat principle will be used, such that all available data from all randomized subjects will be included.

For continuous measures, each dependent measure (Y_j) is predicted by the following model: $Y_j = \beta_{1j} + \beta_2 T + \beta_{3j} T + \beta_4 I + \beta_5 TI + \beta Z + \varepsilon_{ij}$ where I indicates treatment status; j indexes subjects; T indexes time (for repeated measures); β_{1j} is a random effect for the intercept of the jth subject; β_2 is the average slope; β_{3j} is a random effect for the slope of the jth subject; β_4 is treatment status; β_5 is the difference in slopes over time between groups specified in β_4. βZ is the effect of possible covariates (e.g., child's age or diagnosis), and ε_{ij} is a random effect for error. We will assess significance of β_5 (group-by-time interaction) to evaluate effects of sham/active attention training on symptoms. Of note, the Pediatric Anxiety Rating Scale (PARS) will serve as the primary outcome measure in this analysis. However, secondary outcome measures will include measures of self and parent reported symptoms as well as reports of symptoms collected on EMA technology. Secondary analyses will examine moderation of treatment response based on the behavior of the therapist during CBT. This behavior will be quantified whereby the methods from Ewbank et al.[161] will be used to classify therapist behavior. This will require transcription of therapist speech to the patient during therapy and classification of this speech using machine learning to determine if features found to predict CBT response by Ewbank et al. also predict CBT response in the current protocol.

Similar models will be used to test hypotheses on cognitive task performance and physiology in the laboratory-based tasks. For these measures, a continuous outcome variable will be predicted from task-related factors interacting with diagnostic status. For example, in cognitive tasks, reaction time can be predicted from an emotional task factor and diagnostic status, as well as the interaction between diagnostic status and the emotional task factor. Similarly, in fear conditioning tasks, skin conductance can be predicted from conditioning, diagnostic status, and their interaction. Finally, for CO2 inhalation, symptoms on the DSQ or measures of respiratory physiology can be predicted from the timing of CO2 exposure, diagnostic status, and their interaction.

11.4. Data sharing for secondary analyses:

Data collected as part of this protocol will be shared with our co-investigators at NIMH (e.g. Ellen Leibenluft) and outside NIMH, as well as with our collaborators on this protocol. The information may be used for other research purposes including those not related to anxiety disorders or depression.

Beyond processed data, we also will send raw data, for more refined analyses, to a few approved collaborators outside of NIH. Thus, we will send such data to Dr. Amy (Krain) Roy as part of our collaboration with her. Dr. Roy is a collaborator on this protocol, and her role in conducting secondary analyses is part of an ongoing collaboration with her lab. The shared data will be stored in Dr. Roy's lab using the same safeguards currently used by SDAN. We also will send raw data to Dr. Amanda Guyer, who has worked on data when she was a member of Dr. Pine's research group. Dr. Guyer also is a collaborator on the protocol. She has moved to the University of California, and she also will store data on her server using the same safeguards currently used in SDAN. Dr. Fox also will receive raw data. We will also send raw data to Dr. Dylan Gee and Dr. Eli Lebowitz, both at Yale University, who are collaborators on this protocol, to support measures collected at, and data of interest to, both sites. We will also send raw data to Dr. Johanna Jarcho and Dr. Tessa Clarkson, both at Stony Brook University, Dr. Yair Bar-Haim and Ido Tavor, both at Tel Aviv University, Dr. Roxann Roberson-Nay at Virginia Commonwealth University, and Dr. Ineke van der Ham at Leiden University. All are collaborators on the protocol.

Finally, components of the current protocol are being performed in a cross-site study, involing the NIMH, the University of Wisconsin, and Vanderbilt University. To support this work, for measures acquired across the three sites, we will send brain imaging, psychophysiology, and clinical data as well as salivary cortisol, and raw video data from the TSST to Dr. Lisa Williams and Dr. Ned Kalin. Drs. Williams and Kalin are current investigators at the University of Wisconsin and associate investigators on this protocol.

Only if participants agree to let us share fMRI data with the scientific community at large, will we make those fMRI data acquired during resting state available to the overall scientific community. This will be done as part of the 1000 Connectomes project, which is part of the Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC). The 1000 Connectomes Project data sharing effort will provide the research community with open access to datasets contributed by labs around the world. In this instance, all personal identifiers will be removed from the data to maintain subject privacy. Of note, associate investigators will have full access to all data necessary to conduct all analyses. However, personal identifiers always will be removed before data are shared with the scientific community at large as part of the 1000 Connectomes project.

Datasets provided to the 1000 Connectomes Project are to be de-identified prior to deposition of the data with the project (i.e., removal of any personal identifying information from header/support files). Upon arrival, datasets are automatically organized and header files are replaced with novel header files to guarantee that any identifying personal information within the header or supporting files is removed.

Data may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data samples from this protocol may be open-access or restricted access.

The IRB granted a waiver of written informed consent on 6/29/2017 to share individual-level data from the current protocol with a publicly available data base, ENIGMA Consortium (http://enigma.ini.usc.edu/), unless subjects explicitly state that they do not want us to share their data in this manner. This also applies to participants who have opted in to data-sharing, but are missing a witness signature on their consent form. Subjects who have agreed to share their data will be provided pertinent information where applicable. While we will provide group-level data to help this international group, more powerful analyses can be conducted with individual level data, as noted on the Consortium website and papers. Data from all subjects entering the protocol prior to 2018 will be shared with members of the ENIGMA Consortium, unless there is documentation of the subject contacting us to ask that we not share their data. After 2017, consent forms will document whether subjects provide consent for their data to be shared with the ENIGMA Consortium.

This waiver specifically applies to participants with whom we have had no contact since 2018 or who have returned a signed data-sharing consent form without a witness signature. Prior to obtaining a waiver, we heard back from 68 people allowing us to share data. Unfortunately, 48 of them did not have a witness signature, thus these consents are considered “not proper”. Instead of requiring that these participants go through the consenting procedure again, the IRB waived the necessity to reconsent these participants as they had clearly indicated that they would like to share their data. This waiver also applies to all other participants entering this study before 2018 with whom we have not had contact. The regulatory requirements for this waiver have been met.

Requirement 1: The research involves no more than minimal risk to the subjects. Data will not contain identifying information. As a result, there is no more than minimal risk of compromising subject’s identity.

Requirement 2: The waiver or alteration will not adversely affect the rights and welfare of the subjects. For any subject who requests at any point that we refrain from sharing data, we will refrain from sharing their data. This applies to subjects no longer enrolled in the protocol who contact us at any time asking us to avoid doing so. As reviewed with the IRB as part of amendment VVVV in 2017, we attempted to contact large numbers of people from this protocol to obtain updated consent. However, many subjects did not respond to our requests, and it was not feasible to obtain consent from subjects with whom we have had no contact.

Requirement 3: The research could not practicably be carried out without the waiver or alteration. As noted above, many subjects no longer enrolled in the protocol failed to respond to our 2017 requests for updated consents. By excluding these participants, we are limiting the generalizability of our data.

Requirement 4: Whenever appropriate, the subjects will be provided with additional pertinent information after participation. Whenever appropriate, we will provide participants with additional pertinent information. It is unlikely that we will get any additional information, but if we do we will provide that pertinent information to the participant.

Data will be stripped of identifiers and may be coded or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

Prior to open-access sharing via web- based repository, all datasets will be further de-identified and anonymized by the removal of face information from the image to prevent any inappropriate reconstruction of the image that could lead to identification of a participant. Further, each individual participant's dataset will be assigned a randomized five-digit participant identifier, along with a site identifier (two letters which map to the site providing the data). The relationship between the anonymized code and the original subject ID will be destroyed so as to assure that the dataset will be truly anonymized. For each dataset, demographic information provided via web-archive will be limited to (when available): age (18 and up), gender (male, female) and handedness. This information will serve to facilitate more careful characterization of the data, without entailing risk of violation of confidentiality.

11.5. Sample Size Justification:

Determination of sample sizes is based on the primary goals of this protocol, to document neuro-cognitive differences between patients and healthy groups on neuropsychological and fMRI tasks. Sample size determinations are not informed by hypotheses on treatment effects. This derives from two factors. First, it is impossible to determine sample sizes for these contrasts, as they depend partly on the treatment choices of families and on the treatment response rates of subjects. Second, these contrasts must assume that pre-treatment differences in neuro-cognitive and fMRI parameters differ between patients and comparisons. Given the paucity of prior work, this is a somewhat tenuous assumption.

Power calculations assume sample sizes of n=45 for each contrast. This includes groups with specific anxiety disorders, and it includes studies that separately consider adults or youth. Of note, for studies of major depression, the sample size will be larger (n=60) when considering all depressed subjects as a group, either among youths or among adults. However, sample sizes are expected to be smaller for subjects with depression, sub-divided based on the presence or absence of an anxiety disorder (n=30 for each, in adults and in youth).

With sample sizes of n=45, statistical power is adequate (β>0.80) for detecting differences between patients and comparisons, assuming medium-to-large effects (standardized difference [***d***]=0.8-1.2).[96] Such effect sizes have typically emerged in prior studies documenting hypothesized between-group differences in psychological function or fMRI activation. This includes prior studies in both adults and in youths. Moreover, we expect to see similar differences in youths and adults, and our prior studies in both groups find between-group differences of this magnitude. Thus power will be adequate to test all of our primary hypotheses concerning both fMRI and neuro-cognitive differences between patient groups and healthy adolescents as well as our secondary hypotheses on comparable differences in adults. For any comparison where a cell contains less than 45 subjects (e.g. subjects with major depression but not an anxiety disorder), power will be less than adequate. These data will be used to generate estimates of effect sizes to consider the feasibility of conducting further studies in such relatively select subject groups. Finally, for treatment effects, based on prior work in this protocol, we expect a medium effect separating patients receiving CBT randomized to active and placebo forms of treatment. Therefore, the plan is to include 120 subjects in the RCT, with 60 randomized to the active form of ABMT and 60 randomized to the placebo form of ABMT.

12. Human Subjects Protection:

12.1. Subject Selection:

This study examines brain structure and function in psychiatrically healthy as well as clinically anxious or depressed children, adolescents and adults. For healthy subjects, volunteers will be solicited, as prior studies performed at the NIH on developmental aspects of brain structure document the feasibility of this approach.[97, 98] Impaired subjects will be recruited when they present for treatment. Sample sizes for the current project derive from power considerations in the proposed fMRI and treatment studies. In general, power in the proposed fMRI studies will be adequate to detect large effect sizes with n=15. Assuming that acceptable data will be generated from approximately 90% of studies, the proposed sample size will allow us to obtain data in 15 subjects for each relevant group of subjects.

To recruit subjects, families of healthy volunteers will be advised of the nature of the assessments and neuroimaging (fMRI, M/EEG) procedures included. Participants will be informed about the purposes of each research study. Specifically, they will be told that the information derived (a) may eventually lead to better understandings of the associations between brain function and emotional disorders; (b) will utilize a confidential code number to ensure that only associate investigators and their research staff will be able to link information about any person or family to data collected as part of this study; and (c) will be treated to group statistical analyses only. In addition, families of patients will be advised on aspects of the treatment trial. Specifically, they will be told (a) that this study may eventually lead to better understandings of effective treatments for mood and anxiety disorders in youth; (b) that participants may be treated with either an active or placebo attention-retraining procedure in a double-blind fashion; and (c) that families will be removed from the study and provision for alternative treatments will be arranged either when clinically indicated or at any other point that a family so desires. All participants will be provided with a minimum of one-month free treatment following study participation.

12.2. Justification for Inclusion/Exclusion of Children:

The current study focuses specifically on developmental aspects of mood and anxiety disorders. As a result, the protocol includes both children and adults. The tasks in this protocol have not been validated in a language other than English, therefore all subjects will be English speaking.

12.3. Justification for Inclusion of Other Vulnerable Subjects:

As the current study focuses specifically on developmental aspects of mood and anxiety disorder, other vulnerable populations besides children and adolescents are included. Specifically, subjects with ongoing mental disorders are included. Because the current protocol is explicitly designed to generate information on the pathophysiology and treatment of mood and anxiety disorders, people with these conditions necessarily must be included.

12.4. Justification for Sensitive Procedures:

None of the procedures in the current protocol appear particularly sensitive. Nevertheless, any form of research in children with mental disorders, a particularly vulnerable population, requires careful attention to issues of tolerability. Three aspects of the procedures deserve at least some brief discussion.

First, controversy exists on the treatment of pediatric mental syndromes. The treatments offered as part of the current protocol represent the current standard of care in the community. Attention training is also offered to some children in the current protocol, and this is not the current standard of care. However, attention training is not thought to carry more than minimal risk, and attention training will be added to other, standard treatments, either CBT or SSRI medication.

Second, the current protocol involves emotionally-arousing procedures. All of the procedures currently employed in the protocol have been used in more than 50 children and adolescents. Most have been used in far larger samples. Based on these experiences, the level of emotional arousal induced by any of the procedures is commensurate with the level experienced by children and adolescents in their every-day lives. In approximately 10 years of research using such procedures at the NIH, the investigative team has not had a single complaint concerning the upsetting nature of these procedures.

Finally, subjects are exposed to paradigms involving deception. Subjects are notified of this possibility at consent, and subjects are debriefed following study participation. Based on experiences with more than 100 subjects in the current protocol, no adverse effects have been noted. Prior to initiating these procedures that rely on deception, initial studies had attempted to use procedures without deception. These procedures were unsuccessful in eliciting the expected emotional and information-processing responses, as subjects found the procedures minimally engaging.

12.5. Safeguards for Vulnerable Populations:

A number of safeguards are in place in the current protocol.

Protections for NIH employees, staff and family members participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff.

12.5.1. *Recruitment and Assessment Procedures*: To reduce subject fatigue, the recruitment and assessment procedures may be spread out over two visits, with breaks interspersed to reduce any discomfort. In the case of a subject becoming distraught in the course of the assessment procedures or at any other point during the study, staff will pursue steps for appropriate intervention, as outlined above. If, in the opinion of the study staff, PI, subject, or his/her family, the assessment procedures or study participation is adversely affecting the subject's emotional well-being, the clinical circumstances will be reviewed to determine what additional steps should be taken.

12.5.2. *Neuropsychological and Neuroimaging procedures*: We routinely use a series of procedures to minimize the risk for upset in children, adolescents, and adults who participate in our prior and proposed studies. Namely, the procedures are conducted with close oversight of trained clinicians. Each subject is interviewed by a member of the research staff immediately following each procedure. For fMRI studies, this interview is conducted by a credentialed clinician; for tests conducted in the clinic, interviews are conducted by trained research staff. In the event of any level of upset experienced by the subject, an immediate evaluation is performed by available clinical staff. Follow-up assessments are conducted at each clinic visit, conducted following the neuropsychological procedures. Hence, any problems that stem from these procedures will also be detected. All subjects are debriefed following the deception procedures. For the clinic-based procedures, clinical staff is available on-site at all times. For the fMRI procedures, subjects will have ready access to a clinician who will be present at the scan, should they experience any problems. Moreover, through the use of a simulator, subjects will be habituated to the MRI environment. Specifically, during scanning the subject can be seen at all times by a person standing in front of the bore or through the window between the control room and the scanner room. The subject can communicate with the control room personnel via an intercom at the operating console. The subject can be removed immediately from the scanner if necessary. Subjects will wear earplugs to minimize exposure to excessively loud noises, and the length of each MR study will not exceed 90 minutes.

12.5.3. *Treatment:* All subjects are seen on a regular basis by a trained clinician. Moreover, families are given pager numbers for trained clinicians who are available 24 hours per day. As a result, any potential worsening in a subject's condition should be detected very quickly. Such instances of worsening will be reviewed by a panel of clinicians. This review will be designed to remove subjects from the trial who show significant worsening, in an effort to minimize any potential adverse consequences of treatment. There is a possibility that attention retraining could slow a child's response to CBT or fluoxetine. Families will be informed of this possibility upon study entry. There is also a risk that patients could be identified by companies who are hired to create transcriptions of the psychotherapy sessions. This risk is minimized by following procedures delineated by NIMH. These authorities have determined that the risk for such disclosure is minimal as long as the company performing transcription signs a non-disclosure agreement, notifies the study team of incidents of data lost, using a FedRAMP approved cloud system or FISMA approved contractor system rated "moderate" for sensitive data, agrees to destroy residual data, and uses encryption for communication.

12.5.4. *Emergency Treatments:*

12.5.4.1. Assessment Phase: If, during the course of interviewing and assessment procedures, study staff identify a condition that should require immediate clinical intervention or official reporting (e.g., homicidality/suicidality, child abuse), all necessary steps will be taken. In the case that staff determine that the child is at significant risk for self- or other-destructive behavior, the parent will be notified, any mental health or professional persons currently treating the child and family will be contacted (with parental permission), and necessary treatment steps (e.g., hospitalization, referral to a care provider, etc.) will be offered. Parents are advised during the consent process that the law requires reporting of child abuse. For

subjects in the treatment trial, weekly visits with a psychiatrist will occur. This will allow close monitoring of all subjects during treatment.

12.5.4.2. Treatment phase***:*** If, during the course of the study, a subject's condition deteriorates to the point where emergency treatment is needed, the subject will be removed from the trial. At this point or any other point where subjects are removed from the trial for non-emergent reasons, clinical care will be provided by staff within the Mood and Anxiety Disorders Program, under the direction of Drs. Pine and Zarate. This care will continue until the case has been successfully stabilized, though the use of standard clinical practice in the treatment of pediatric mood or anxiety disorders. If inpatient hospitalization is required, this will occur at the Clinical Center at NIH in the unit directed by Dr. Zarate.

12.5.4.3. Clinical Care After Treatment***:*** The total amount of time that out-of-study care will be provided is expected to vary, given the diverse clinical needs of patients. Ideally, the team will provide care for between one and two months in most cases. Such a level of care would be expected for all children, including children who do well in the trial. However, efforts to transition care to the community will intensify after three months of treatment have been provided. The study team will make every effort to ensure that no more than six months of treatment are provided.

13. Benefits:

There is potential for direct benefit to participants who receive treatment with fluoxetine or with CBT. For healthy volunteers, there is no direct benefit to the individual subject. For all participants, this research may yield generalizable knowledge about the causes and treatment of anxiety or depression in children and adolescents. Such information could eventually allow clinicians to better prevent and/or treat this disorder. Moreover, for subjects with disorders, subjects will receive direct benefit in the form of treatment.

14. Consent Documents and Process:

Study investigators will obtain informed consent, as described in the Study Personnel Page.Assent will be obtained from minor subjects. Consent for this protocol will be obtained from parents/legal guardians. Of note, subjects who enter the protocol as 17-year-olds will be reconsented as adults if they turn 18 during the course of the study.

If the parents are married, written consent may be obtained from one parent only. If the parents are not married, written consent will be obtained from: 1) the custodial parent if only one parent has legal custody, or 2) from both parents if they share legal custody for medical decision-making. For unmarried parents, signature of one parent will suffice if the other parent is deceased, unknown, incompetent, or not reasonably available. When signature of both parents is required, written consent will be obtained in-person from at least one parent. When the other parent is unable to attend the consent process conference in person, the following telephone process to obtain written consent will be used.

Telephone Consent Procedures: The unavailable parent will be provided with a copy of the consent form, usually by fax, email, or hard copy mail. Once the consent form is received, an Investigator authorized to obtain consent will arrange for a telephone call with the parent to review study and the consent form and to answer any questions. Once the parent agrees to his/her child's participation, the parent will sign and date their copy of the consent form. The Investigator will enter a note documenting the consent process in the Medical Record. The parent will return their signed copy to the Investigator. Once the copy with the parent signature is received, the Investigator will sign and date the telephone-parent consent form, place the original

copy with all signatures in the Medical Record, retain a copy for research records, and mail a copy to the parents.

For participants that do not feel comfortable doing in-person research visits/encounters, telehealth will be used to obtain informed consent for participation in research and we will comply with all requirements of Policy 3014-301 Informed Consent. For telehealth informed consent visits, the informed consent documents will be provided to the potential participant in advance of the telehealth encounter in an electronic format via the Secure Email and File Transfer Service (SEFT), mail, or iMed.

Participants will be provided blank consent and assent forms either via SEFT,mail, or iMed in advance of the consent date so that they are given an opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. For in-person consents, once the consent forms are received by parent, an Investigator authorized will arrange for obtain consent will set an in-person consent date. For phone consents, once the consent forms are received by parent, an Investigator authorized to obtain consent will arrange for a telephone call with the parent to review the study and the consent form and to answer any questions. A telephone consent date is set.

For telehealth consents, once the consent forms are received by parent, an Investigator authorized to obtain consent will arrange for a consent visit to be scheduled via the "Virtual Visit Electronic Appointment Request" in CRIS.

On the consent date and time, the investigator authorized to obtain consent reviews the consent forms with parents and child present either in-person, on the phone, or via telehealth in a language that they understand and answers any questions both child and parents may have. After consents have been reviewed, all questions are answered, and parent and child are fully informed of the study, parent and child are asked to sign and date the consent. Parent and child sign and date the consent forms.

For telephone and telehealth consents, parents and children that have printer and scanner capabilities print the consent forms sign and date by hand and then scan them and return to investigators via SEFT. Some families don't have printer and scanner capabilities, these parents and children sign the consent using a mouse, stylus or touchscreen or use the Sign icon  in the toolbar of Adobe. Using this icon, they add their signature using the *add your signature* feature and *draw* their signature and their names on the consent and date the consent. The signature MUST be provided using this feature otherwise it is returned to the parent and child to sign using these features. The iMed platform, which is 21 CFR, Part 11 compliant, may also be used for telephone and telehealth visits to obtain the required signatures. During the consent process, participants and investigators will view individual copies of the approved consent document on screens in their respective locations. Both the investigator and the participant will sign the document synchronously with a hand signature using a finger, stylus, or mouse.

For in-person consents, the investigator signs and dates the consents. For phone and telehealth consents, the parent will return their signed copy to the Investigator. Once the copy with the parent signature is received, the Investigator will sign and date the consent form, place the original copy with all signatures in the Medical Record, retain a copy for research records, and provide a copy to the parents. The Investigator will enter a note in CRIS documenting the consent process in the Medical Record. When the consent is obtained via phone or telehealth, the Investigator entering the consent documentation will specify the modality.

For participants who were enrolled prior to the addition of broad sharing, we may obtain written consent for sharing of de-identified data via a telephone process similar to the one described above. If the participant is

still an adolescent, the process above will be followed with the consent, and if consent from both parents is required, the written consent from both parents may be obtained via this telephone process.

If a minor participant has reached the age of consent and is not reasonably available for consent so that the research could not be practicably done without the waiver, the use of samples and data collected with parental consent, may continue under a waiver of informed consent. As state stated below, we have met the regulatory requirements under 45CFR46, to be granted a waiver.

Requirement 1: The research involves no more than minimal risk to the subjects.

The continued use of data already collected poses no more than minimal risk.

Requirement 2: The waiver or alteration will not adversely affect the rights and welfare of the subjects.

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

Requirement 3: The research could not practicably be carried out without the waiver or alteration.

The research cannot be done without this data. In our experience, getting consent is not practical given the number of subjects and time that has passed.

Requirement 4: Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

Whenever appropriate, the subject will be provided with additional pertinent information after participation.

Consent for NIH employees and staff will not be obtained by coworkers.

Request for waiver of documentation of informed consent for <u>collection and use of data</u> from new participants consenting during COVID-19 pandemic

We will recruit participants to participate in our longitudinal study of anxiety (i.e., this current protocol) to consent them if they meet our inclusion criteria and are interested in participating in our research. If the participant is interested in participating, we will attempt to consent them using the standard telephone consent procedure and the telehealth consent procedures reviewed by the Health Information Management Department. If the participant or parent / legal guardian is unable to adhere to the documentation of consent procedures (i.e., cannot return a signed copy of the consent document due to technological limitations), the collection and use of data with parental consent and, if applicable, minor assent may continue under a waiver of documentation of informed consent. This waiver of documentation of informed consent is appropriate because:

1. The only record linking the subject and the research would be the informed consent form, and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject (or legally authorized representative) will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern;
2. The research presents no more than minimal risk to subjects and involves no procedures for which written consent is normally required outside of the research context; and
3. Once it is deemed safe for subjects to come on site to participate in our research, we would invite subjects (for whom we waived documentation of informed consent) on site. For subjects who are

willing and able to come on site, we will obtain written documentation of informed consent on site. Once we have obtained a written documentation of informed consent, the consenting clinician will record that written documentation of informed consent has been obtained by writing a note in the secure Clinical Research Information System.

We have prepared a written script that investigators will follow that describes the information that we will verbally convey when we review our consenting process with participants. This script also includes information on how we will record this discussion with participants by writing a note in the secure Clinical Research Information System. This script has been uploaded as an attachment in iRIS.

When consenting participants, we will ensure that participants are able to view any relevant consent and assent forms during consenting by sending them the most up-to-date versions of our study's consent and assent forms for them to reference prior to the consenting phone call using Medical Secure Email.

Subjects thus far have experienced numerous difficulties when attempting to return the signed consent document to NIH staff. Some subjects have been unable to return the consent document due to lack of access to a printer and scanner. Other subjects have been unable to return the consent document due to various technological limitations (i.e. certain subjects do not have Adobe Acrobat, do not understand how to download Adobe Acrobat, or do not understand how to use Adobe Acrobat to sign a document). Our staff have experienced a low success rate when working with subjects to return the signed consent document to NIH staff purely due to technological difficulties.

Subjects cannot pursue other means of returning the consent document to NIH staff (such as using pre-paid US mail envelopes to return the signed document) because this would require NIH staff to come onsite to mail subjects pre-paid US mail envelopes and to retrieve those envelopes upon return of the signed consent document. We have received guidance from the NIH Division of Occupational Health and Safety (DOHS) to maximize use of telework and flexibilities in order to prioritize the health and safety of our staff given the current COVID-19 pandemic. In accordance with these guidelines, our staff are maximizing use of telework and are thus not positioned to mail subjects pre-paid US mail envelopes and to retrieve those envelopes. Most of our staff has not yet received approval to return to their physical workspaces, and thus would not have the resources available to mail subjects pre-paid US envelopes to return the signed consent document.

15. Data and Safety Monitoring:

Data and safety will be monitored by the Principal Investigator, working closely with Associate Investigators.

Dr. Carlos Zarate will serve as the Data Safety Monitor for this protocol. Dr. Zarate will work closely with the research team, but he will not be assigned to provide treatment for patients enrolled in the protocol. Dr. Zarate will also review each case where there is a question of the need to remove a case from the trial. As the Data Safety Monitor, Dr. Zarate will have the authority to remove a child from the trial, irrespective of the decision made by the research team. Dr. Zarate will, however, remain blind. This decision results from the fact that we feel that identical criteria must be used to remove subjects from the trial, regardless the treatment the subject is receiving. This is a decision that is based on clinical interests of the patients, in that patients should not be in the protocol, no matter the treatment, if they demonstrate clinically significant levels of deterioration that pass a pre-specified threshold. By removing the blind, one might introduce a bias, whereby subjects may be more likely to remain in the trial on one or another treatment. Dr. Zarate will have

the authority to remove independently a child from the study based on clinical grounds, as determined by his evaluation.

16.1. Healthy Subject: For healthy subjects, the study involves assessments, two imaging sessions, and no experimental therapies. These subjects will be monitored by a mental health professional. Subjects will be monitored by other staff (e.g. IRTAs) during non-imaging procedures, with professional staff supervising. In all subjects undergoing scanning, procedures will be stopped for any subject who asks to stop any procedure at any point. Subjects will be asked if they wish to continue with other phases of the study. Procedures will also be stopped for any subject who exhibits signs of distress during any phase of the study, even if they do not request stopping. At this point, subjects will be interviewed to confirm that they are in fact in distress. Subjects will be asked if they wish to continue with other phases of the study.

16.2. Patients:

16.2.1. *Monitoring Procedures*: For psychiatrically impaired subjects, the current study offers three to twelve weeks of treatment, with additional visits offered as they transition to the community. Clinical response will be monitored weekly by a psychiatrist or psychologist throughout the study during in-person visits. Families will be provided with phone numbers for emergency coverage, 24-hours/day. Clinical response will be rated on the CGI-I Scale. This scale incorporates all clinically relevant data that is gathered during the trial. This includes data from the subject, parents, or other sources, as well as data from the clinician's observations. As a result, the assessment of clinical response, as rated on this scale, will comprehensively capture the global clinical state of the subject and the need for intervention, should patients begin to deteriorate. In addition, adverse events associated with medication will also be monitored on a weekly basis. A summary of all adverse events will be provided to the IRB during each yearly review of the protocol.

16.2.2. *Procedures for Removing Subjects from the Trial*: Subjects will be removed from the study by the research team in three circumstances. First, in each instance where a subject shows clinical worsening or clinically significant adverse events, details of the case will be reviewed independently by three clinicians during the first visit where the subject shows worsening as well as the next visit. Results of this review will be discussed with the patient and families. Clinical worsening is defined as a rating of "7" ("worse") or greater on the CGI-I for any visit. Subjects will be removed from the study if they are rated as "worse" by two of the three clinicians for two consecutive weeks, though families are free to discontinue the trial at any point. Second, subjects will be removed if they exhibit clinically significant increase in suicidal ideation. Third, Dr. Carlos Zarate will serve as the Data Safety Monitor for this protocol. Dr. Zarate will work closely with the research team, but he will not be assigned to provide treatment for patients enrolled in the protocol. Dr. Zarate will also review each case where there is a question of the need to remove a case from the trial. As the Data Safety Monitor, Dr. Zarate will have the authority to remove a child from the trial, irrespective of the decision made by the research team. Dr. Zarate will, however, remain blind to treatment status for subjects enrolled in the double-blind phase of the treatment protocol. This decision reflects the fact that identical criteria should be used to remove subjects from the trial, regardless of the treatment that subjects receive. This is a decision that is based on the clinical interests of the patients, in that patients should be removed from the protocol, no matter the treatment, if they demonstrate clinically significant levels of deterioration that pass a pre-specified threshold. By removing the blind, one might introduce a bias, whereby subjects may be more or less likely to remain in the trial on one or another treatment. This could increase the chance that subjects are removed from the trial due to bias, as opposed to purely on clinical grounds related to their current status.

16. Quality Assurance

16.1. Quality Assurance Monitor:

Quality assurance will be monitored by the Principle Investigator, Dr. Daniel Pine, the research team and the NIMH Office of Regulatory Oversight (ORO).

16.2. Quality Assurance Plan:

ORO monitors intramural research studies to ensure compliance with GCP, organizational policies and regulations. Audit frequency is determined by the IRPAC SOP based on the study level of risk. Results of ORO audits are provided to the PI, The Clinical Director and the IRB.

This study will undergo audits at least once every three years and for cause.

17. Adverse Event Reporting:

Reportable events for this protocol will be tracked and reported in compliance with Policy 801.

18. Alternatives to Participation:

For healthy volunteers, no treatment is provided in this study, and these subjects will not forego any treatment in order to participate in this study. For these subjects, therefore, the alternative is not to participate.

For patients, the treatment provided in this study is the same as the treatment that can be obtained in the community. Therefore, for these subjects the alternative is to receive treatment in the community.

19. Privacy:

All research activities will be conducted in as private a setting as possible.

20. Confidentiality:

Every necessary step will be taken to prevent identification of study participants or violations of confidentiality of the data. Information will be stored using a confidential code wherever possible and data will be treated only as groups. Data on clinical status will be stored in CTDB, and data related to pathophysiology collected as part of this protocol will be stored in the Neuroinformatics Database (NiDB) (http://nidb.sourceforge.net). Of note, to properly link data between CTDB and NiDB it will be necessary to include data for the name, sex, and date-of-birth of subjects both in CTDB and NiDB. However, the plan for maintaining confidentiality in each of these databases has been reviewed with William Hermach from the Information Resource and Technology Management Branch, and he has determined that procedures for data storage meet NIH security standards. Access will be restricted to approved investigators and staff, and we will utilize the same protections currently in place for CTDB. All data entered into the NIH CTDB will appear only in coded form, though, when necessary, subject identity will be available to appropriate staff. The coded data entered into the NIH CTDB will also be transferred and stored in The Neuroinformatics Database. However, members of the research team and their associate investigators will

have access to these coded data and to information linking coded data to information about specific individuals.

Beyond the procedures noted above, all other information will be stored using a confidential case number, and no identifiers (name, address, phone number, etc.). Thus, unless personnel have access to these identifiers encrypted in the data base, it will not be possible to directly link CTDB information and The Neuroinformatics Database information to individual subjects. Where temporary linking of information with identifiers is needed, such identifiers will be temporarily attached to the data, and will be removed after information has been encoded. Information will be stored using only a confidential code whenever possible, and data will be treated only as groups. All data entered into CTDB will appear only in coded form. Members of the research team will have access to these coded data. Only staff directly involved in the care of each subject or who need to confirm the identity of subjects containing data within different databases will have access to documents that contain identifying information. This will include research staff, clinical staff, and the study psychiatrist.

Secure laptops will be provided to subjects for the attention retraining sessions. These laptops will not be used to collect personally-identifying information and will not contain any personally-identifying information. Subjects will not be able to use the laptops to access NIH networks. The laptops will be encrypted and will have a general user account that only permits the user to execute programs. The Internet will be locked out until the laptop returns to the NIH. All games will be removed from the laptops and no such software will be able to be installed.

We are planning to use 6-8 laptops for this project. Although we cannot predict how many laptops will be used at home by the subjects at a given time, at most, 6-8 laptops will be in use for the purposes of this study. During the course of the project, the subjects will be visiting the NIH on a weekly basis to participate in CBT treatment. If a subject is discontinuing CBT treatment before the end of the eight week period, the laptop will no longer be sent home with the subject. We will follow standard NIH policies with regards to a lost or missing laptop. We will instruct subjects on these policies and ask subjects to notify us immediately if the computer is lost or stolen.

Before each subject is sent home with a laptop, the machine will be cleaned of any residual data. Therefore, any data that is collected during the course of the study will correspond to the current subject that is participating. The subject will receive a unique de-identifiable login. The subject will run an executable file that will save data to the hard drive according to another unique de-identified subject code. The data will be retrieved by a member of the research team each week at their regularly scheduled CBT treatment sessions.

In using the online data collection for the DAWBA, our procedures minimize the risks to patient confidentiality in the following ways:

1. No personally identifiable information (PII) is collected. Patients and their parents/guardians are explicitly instructed not to use any such information when answering questions.
2. Only our team at the NIMH, but not the web team or any other party, can identify the person providing the information or who the information concerns. The only people with access to the key that links the patient name with the password provided to the patient are in our lab group. That key is stored on our secure server at NIH.
3. The website on which the data are collected is secure and has been used successfully around the world to collect such data, including in Scandinavian countries with strict regulations on patient confidentiality commensurate to the ones applied by the NIH. It has passed the Privary Impact Assessment (TPWA-PIA) by our ISSO, Mr. Hermach, at NIH.

4. The founders and owners of the website, Professor Robert Goodman and Professor Michael Smith have an established track record with this method of data collection and enjoy a worldwide reputation for top academic work and commitment to confidentiality through IT applications. They have worked with a number of governments around the world, are known to our group, and are long-time trusted collaborators with Dr. Stringaris prior to his coming to NIH.
5. DAWBA forms that have not been downloaded to our server, and thereby removed, will be purged from the web application within two months of their completion.

21. Conflict of Interest:

None

22. Technology Transfer:

None.

23. Research and Travel Compensation:

All families will receive reimbursement based on NIH standards for time devoted to the research project. Patients will not receive reimbursement for participation in the treatment study, but they will be compensated for participation in other non-therapeutic aspects of this study at the same rate at which volunteers will be compensated. Hence, both patients and volunteers will receive reimbursement for participation in the neuropsychological, psychophysiological, MEG and fMRI procedures. This compensation will be used to defray days lost from work, baby-sitting expenses, etc. Subjects will be compensated for their time for each procedure.

Payment Schedule: Subjects will be compensated based on the schedule shown below. This compensation will be provided for each of the times that the subject completes the procedure, though compensation for the psychiatric assessment will only be provided at the initial assessment. Therefore, families of children/youth who complete all phases of this study will receive up to $940, depending on whether computer tasks, MEG scans, and fMRI procedures are completed twice. Adult patient participants receiving open treatment will be reimbursed $320. Additional compensation is provided if monetary tasks are completed. In this protocol there are tasks that pay participants based on their winnings. The three reward tasks from work by Averbach (Shopping, Novelty, Horizons), Affective Posner 2, MID, and PRR pay participants up to $ 25 (for each task) more than the amount for just performing the task; the extra amount is based on their winnings during that task. The total amount of winnings from all these tasks (whether outside or inside the MRI scanner) that any participant can receive will not exceed $300.
Of note, participation is lower for follow-up relative to baseline measures. One set of follow-up measures includes measures of symptoms assessed through questionnaire. This to generates measures of symptoms other than our primary outcome measures for our clinical trials. The other set of follow-up measures includes measures of brain function, to quantify post-treatment changes in biomarkers through imaging.

Based on communication with healthy subjects, at baseline, it appears as if this group of subjects works to arrange their study participation at a time in their yearly schedule where the subjects are relatively free to participate in research. Six to 10 weeks later, when follow-up measures are sought, it appears as if the schedules of many healthy subjects have change, making the follow-up assessments less convenient. Similar changes appear to occur in patients, based on communication with this group. Moreover, at follow-up assessments, patients typically are much improved in terms of their symptoms. As a result, not only are follow-up tests less convenient, but they also come at a time when motivation for participation might

diminish. We add a $100 bonus to be paid after the follow-up assessments are completed, after the follow-up imaging session. Employees and staff who participate during work hours must have permission from their supervisor. NIH employees and staff must either participate outside of work hours or take leave in order to receive compensation.

Task	Total $$	Notes
	As of June 2022	
History	$15	
Phlebotomy	$15	
Cheek swabs	$8	
Initial K-SAD	$30	•
KSADS (Repeat)/SCID	$8	•
Questionnaires	$30	Ex: Demographics, CCC, SRC, MASC
Ratings	$30	Such as the sum of PARs, CDRS, YMRS, BPRS, etc
Six-Month Follow Up Interview and Forms	$30	Ex: PARs
Ecological Momentary Assessment (EMA) - Study 1	$90	Subjects will receive $90 for each time that they return the phone and an additional $10 for responding to 75% of questions. This will be completed three times.
Ecological Momentary Assessment (EMA) – COVID-19 Study	$120	Subjects will receive $20 for a retraining of the EMA protocol, $90 for completing the session, and an addition $10 for responding to 75% of the questions.
Neuropsychological Testing	$40	
WASI only	$15	
Computer Tasks (no winnings) (in-person and mobile)	$15-30	This is given upon the completion of tasks. Payment varies depending on task length (for tasks with monetary incentives, see below)
Computer Tasks (with winnings) (in-person and mobile)	$60	Subjects will receive $40 for completing the task. Extra amount up to $20 based on winnings,
Mobile App Task	$40	Subjects will receive $20 per play, with the opportunity to play twice for a maximum of $40.
Psychophysiology	$15	
Conditioning Experiment (in-person and mobile)	$100	
Heat Pain Task	$100	
Memory Task (in-person and mobile)	$30	
Confidence Task (in-person and mobile)	$10	Extra amount up to $20, based on winnings
Risk and Ambiguity Task (in-person and mobile)	$10	Extra amount up to $100, based on winnings
MID/Knutson (fMRI & behavioral)	W (1 run)	W= 1 run, which subjects randomly pick at the end (subjects should not receive money from the practice run)
WOF	W (1 run)	
PD (behavioral & fMRI)	W (1 run)	W = 1 of 4 runs, which is randomly selected at the end
Eye Tracking (behavioral & fMRI) Antisaccade Task	W (1 run) W (cum)	W= 1 run, which subjects randomly pick at the end

Saccade Task		W=cumulative winnings * pay a minimum of $15 for winnings (as of 7/`4/05) * pay a minimum of $30 for tasks at or exceeding 30 minutes (as of 2/13/19)
∑ winnings < $150 If monetary tasks are completed within a 1 month period, the maximum earnings for each subject is $150. If the time interval between each task is greater than 1 month, do not add the previous gains on monetary task to winnings.		
Functional MRI • • Task: Winnings • Task: Extended time	$130 + W (1 run) $250	Subjects participating in the University of Wisconsin-Madison research project will be compensated for extended time in scanning suite for fMRI.
Structural MRI	$40	
M/EEG scan	$130	Extra amount up to $25, based on winnings
Completion Bonus	$100	
Escort Fee*	$20	Given at the request of parent/discretion of IRTA for family in need, baby-sitting, etc.
Travel Fee	$0.58/mile	Participants will be reimbursed $0.58 per mile for travel by bus, train, or taxi to the NIH.

In regard to total possible compensation, we have summed payments across all research activities, including behavioral tasks and scans, considering all possible repetitions. We have determined that the maximum possible compensation for child participants is $2500 and $1000 for adult participants. This has been clarified in section 23 of the protocol.

24.
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