Specific Aims

The diagnostic system that is currently used for mental disorders is based on verbal report, observable behavior, and clinical phenomena that have been aggregated based on statistical approaches to provide reliable categories[1]. However, the connection between psychiatric disorders and their underlying neurobiology has been difficult to establish. The NIMH Research Domain Criteria (RDoC) framework is a heuristic approach to integrate neuroscience with psychopathology[2]. Thus, RDoC is the attempt to develop constructs that (a) connect across units of analysis, (b) are applicable across established diagnostic criteria, and (c) are a point of departure to provide measurable constructs to gain a deeper understanding of psychiatric conditions[3].

We focus on the positive and negative valence system domains proposed by the RDoC[4,5], using self-report, behavior, physiology, and neural circuit unit of analysis measures, apply them to a clinical population of individuals with anxiety or depression recruited from primary care clinics across two sites and apply latent variable analysis to derive latent constructs of positive and negative valence systems functioning that cut across units of analyses. Our preliminary studies provide initial evidence for the relative independence of measures designed to assess each domain. Building on these studies we will adopt a three stage approach: first, principal component analyses will be used for variable reduction within a task; second, confirmatory factor analyses will be used to determine whether the model of positive and negative valence systems fit with the measures obtained from multiple units of analyses; and third, latent variable analysis will be used to relate measures across different units of analysis. The specific aims of the proposal are:

(1) To recruit, assess, and analyze self-report, behavioral, physiological, and neural circuit unit of analysis data in an exploratory sample of n=100 treatment seeking anxious and/or depressed individuals presenting to primary care clinics at UCSD and UCLA.

a. Hypothesis 1: Measures of reward sensitivity, attentional bias towards positive valenced stimuli, approach behavior in an approach/avoidance task, propensity to display a happy facial expression, increased respiratory sinus arrhythmia (vagal tone), and activation in the ventral striatum during a reward processing task will load together on a latent construct ("Positive Valence System").
b. Hypothesis 2: Measures of high harm avoidance, attentional bias towards negatively valenced stimuli, avoidance behavior in a approach/avoidance task, propensity to display a negative facial expression (e.g., sadness, fear), increased heart rate and skin conductance while viewing negative stimuli, and amygdala activation during a fear conditioning task will load together on a latent construct ("Negative Valence System").
c. Hypothesis 3: Latent variable scores of individuals with anxiety or depression will span a two-dimensional space according to these two latent constructs and will show significant variability across DSM IV (or DSM-5) diagnostic categories.

(2) To obtain a confirmatory sample of n=100 subjects at both sites using the same measurement approaches to determine the reproducibility and robustness of the latent constructs obtained from the exploratory sample.

a. Hypothesis 1: Latent constructs of positive and negative valence systems show good reproducibility in a confirmatory factor analysis.
b. Hypothesis 2: Variable reduction can be used to obtain reliable projection onto the latent constructs using latent variable analyses.
c. Hypothesis 3: Individuals with clinically severe and impairing anxiety or depression will fall into heterogeneous clusters of dysfunctions within the positive and negative valence systems.

(3) **To obtain a reliability sample of n=50 individuals at UCSD and UCLA that will be re-tested within a month to determine the reliability of the latent constructs**.

a. Hypothesis 1: Latent constructs of positive and negative valence systems have good test-retest reliability.
b. Hypothesis 2: Latent constructs of positive and negative valence systems have better test-retest reliability than measures from individual units of analyses.

This will be the first time neural systems measures of positive valence and negative valence systems are investigated within the same broadly defined sample of anxious or depressed patients. Thus, this study has the potential to substantially improve our understanding of how the positive and negative valence systems underlie clinically severe and impairing anxiety and depression in terms of inter-relationships across units of analyses. Upon completion, we will have robust and reliable dimensional measures that quantify the positive and negative valence systems. The latent constructs will be the deliverables of this proposal. We will create a web-based repository to make these latent constructs, i.e. the measures and the derived factors, available to other researchers. These latent constructs will serve as the platform for subsequent work focused on treatment, which can be developed to remediate the dysfunction in the specified dimension(s).

Research Strategy

A. Significance

The Research Domain Criteria (RDoC) framework is a heuristic to integrate neuroscience approaches with psychopathology[2] that will guide the development of a dimensional classification of mental disorders based on genetics and neuroscience[3], however, the lack of reliable, robust latent constructs derived from experimental measures is a critical barrier to organizing mental disorders along dimensions. The RDoC initiative highlights two important goals that need to be accomplished to overcome this barrier. First, one needs to determine the relationship between different units of analyses, that is, between self-report, behavior, physiology, and neural circuits and clinically relevant psychopathology. Second, one needs to transcend traditional diagnostic groups to adequately capture the variation of domains in clinical populations that can be mapped across units of analyses. The current application aims to address these needs.

We propose to use a clinical population of primary care patients with identified problems of anxiety and/or depression for this investigation to maximize generalizability and external validity. Anxiety and depression are the most common mental disorders, and primary care remains the *de facto* mental health service system in the US[6, 7]. By studying patients in this setting, we gain access to the most generalizable sample of persons whose anxiety and/or depressive symptoms are troubling enough that they seek care, without the filtering and biasing that would be seen in general psychiatric or other even more specialized clinical settings (e.g., anxiety or mood disorder clinic). Although we start with well-established measures that are heuristically aimed at quantifying positive and negative valence systems, we expect that the precise mapping onto latent constructs that form the axes for positive and negative valence domains will be more complex and possibly surprising than expected. For example, variables of attentional bias for negative emotion, degree of avoidance behavior in an approach/avoidance task, propensity to display negatively valenced facial expressions, and degree of amygdala activation during a fear conditioning procedure are hypothesized to relate to the negative valence domain. However, the precise relationship and the common variance shared among these units of analysis as well as the most sensitive variables contributing to the domain are fundamentally unknown at this point in time. Once identified, however, this understanding will change the way we conceptualize the positive and negative valence domains, measure them, incorporate them into computational and neural process models, and eventually incorporate them into treatment models.

Once the proposed aims are completed, we will have a robust and reliable dimensional set of variables, obtained from two clinical sites, that quantify the positive and negative valence domains based on a latent variable approach. These variables will subsequently be used to determine whether (a) they predict response to pharmacological or behavioral treatment; (b) they can be differentially responsive to therapies that are specifically aimed at modifying the positive or negative valence systems, and (c) they can be used in subsequent computational and/or neural process models of anxiety and depression to gain a more fundamental understanding of the pathology.

Overview: Positive and Negative Valence Systems

Affect, or the tendency to experience a given emotion, often is subdivided into two domains[8]. Positive affect is the tendency to experience positive emotions, such as happiness, excitement, elation, and enthusiasm. Negative affect is the tendency to experience negative emotions, such as anger, resentment, sadness, anxiety, and fear. Humans exhibit a range of emotions that span across positive and negative affect domains with some individuals experiencing more of one type of affect than another. Positive affect and negative affect systems represent dimensions of psychopathology identified by the RDoC work groups[4,5]. High negative affect is common to anxiety and depression[9-11] and comorbid anxiety and depression is associated with more negative affect than each disorder alone[12]. Low positive affect is relatively specific to depression, with some evidence for social anxiety as well[9,13]. In addition, psychophysiological and neurobiological data indicate that the negative affect system is closely tied to threat sensitivity whereas the positive affect system is closely tied to reward sensitivity.

Positive Valence System: A central construct representing the positive valence system is approach motivation, which can be defined as processes that regulate the direction and maintenance of approach behavior, albeit moderated by pre-existing tendencies, learning, memory, stimulus characteristics, and deprivation states. The construct of reward seeking and reward sensitivity is one component of approach motivation. Reward sensitivity refers to sensitivity to the anticipation and receipt of positive stimuli. The neural mechanisms of reward sensitivity involve the ventral striatum (VS) and orbitofrontal cortex (OFC). The structures are involved in the processing of primary rewards, such as pleasant tastes[14], smells[15] or sights[16], as well as secondary (monetary) rewards[16-18]. The VS plays an important role in the anticipation of reward[19,20] as well as the receipt of reward[17,21]. The VS is part of a larger fronto-striatal circuit subserving reward-related processing that also includes the orbitofrontal cortex (OFC), a subregion of the prefrontal cortex[22]. An

important functional coupling exists between the VS and OFC[23]. Reward-processing also involves other neural regions, including the amygdala[24-26], dorsal ACC[27] and the hippocampus[28].

***Relationship between reward sensitivity and the positive valence system*:** There is evidence to show that individuals with deficits in positive affect (i.e., individuals with depressive disorders) show deficits in reward processing, at both behavioral[29] and neural levels[30]. At the behavioral level, individuals with major depression are less responsive to reward-relevant stimuli than are non-depressed individuals and deficits in reward responding are associated with deficits in positive affect or the ability to experience pleasure[29,31]. At the neural level, depression is associated with attenuated activation in fronto-striatal circuits, namely the VS and caudate, during reward processing[30]. Anhedonia[32,33] (or, the inability to experience pleasure), and reward-related processing[34] have been considered critical factors in the development of depression. Reward sensitivity in anxiety disorders has been less well-studied. Similar to depression, evidence of attenuated striatal activation during reward processing has been found in individuals diagnosed with posttraumatic stress disorder (PTSD)[35,36], particularly in relation to anhedonic features of PTSD (e.g., emotional numbing). Other studies, however, find evidence of heightened striatal activation during reward anticipation in some anxiety disorders[37]. This heterogeneity underscores the potential value of moving towards a dimensional understanding of reward sensitivity and positive valence system functioning in anxiety and depression.

Negative Valence System: Responses to acute threat (fear) and potential harm (anxiety) were considered by the RDoC workshop committee to be central constructs within the negative valence system. One approach to measuring response to threat is via *fear conditioning*, which involves excitatory learning of CS-US associations[38,39]. Research on fear learning uniquely adapts to translational neuroscience contexts because we understand with great precision the relevant neural processes in many species, including humans. The brain regions that have most consistently been associated with fear conditioning are the amygdala[40-44] (AMYG) and insular cortex[45]. In healthy adults, increased activity in the AMYG and insula is typically observed in response to the CS during conditioning. Response to loss was cited by the RDoC committee as another critical component process of the negative valence system, and may be particularly related to depression. Reward paradigms that include loss or punishment trials (e.g., losing money for incorrect responses[46-48]) can be used to measure behavioral and neural responses to loss anticipation and outcome. Research in healthy adults suggests that the ventral and dorsal striatum (caudate) are associated with anticipation and receipt of loss or punishment using these paradigms[46,47].

***Relationship between fear conditioning, loss, and the negative valence system*:** Individuals with disorders characterized by elevated negative affect (i.e., individuals with anxiety disorders), exhibit behavioral and neural perturbations during fear conditioning. In terms of behavioral data, individuals with high trait anxiety or with anxiety disorders show elevated excitatory learning (during fear conditioning)[49,50]. In terms of neural data, individuals with anxiety disorders show elevated AMYG activation during fear conditioning: this has been observed in individuals with posttraumatic stress disorder[51,52], spider phobia and social anxiety disorder[45]. Although no studies have evaluated fear conditioning as a function of depression, other evidence shows that depression as a disorder is associated with dysregulation in the same neural regions that are central to fear conditioning. For example, a number of studies implicate AMYG hyperactivity in major depression during emotion-related tasks such as when viewing fearful faces[53,54], and in individuals with the short allele of the 5HTTLPR serotonin transporter polymorphism[55], which is associated with depression vulnerability[56] and symptom severity[57]. Studies using behavioral paradigms to measure response to loss find that individuals diagnosed with major depression differ from healthy controls in their neural responses to loss in the striatum[58] and anterior cingulate cortex[48]. Some evidence suggests that anhedonic features of depression may be particularly associated with neural activation in the striatum in response to loss[58].

Positive and Negative Valence Systems: Physiological, Behavioral and Self-Report Units of Analysis

To more completely characterize positive and negative valence system constructs, the RDoC initiative underscores the need to (1) identify measures at multiple units of analysis (e.g., self-report, behavior, physiology) that reliably capture the variation of a given domain, and (2) to establish the relationship between different units of analyses, with an emphasis upon linking underlying brain function (e.g., neural circuits) to behavior. Thus, we will use well-established physiological, behavioral, and self-report measures as the units of analysis, and with which our group has prior experience (see Approach section below), to (1) examine latent constructs of positive and negative valence domains and (2) examine the relationships between those units of analysis and neural indices of reward sensitivity and fear conditioning. Our choice of measures was guided by empirical data for specific measures heuristically aimed at quantifying each domain, and evidence suggesting that those measures relate to neural circuits governing positive and negative valence system functioning. For example, attentional biases for emotional information are considered a hallmark of anxiety and depression[59,60]. Attentional bias for threat-relevant stimuli is reliably associated with negative affective states (e.g., anxiety[60]) and has been linked to greater activation in the AMYG during emotion processing[61]. In contrast, attentional bias for positive stimuli is associated with positive affective states[62] and neural activity reflecting approach-

oriented motivation[63]. Moreover, diminished attentional processing of positive information is associated with depression[64], and some anxiety disorders (e.g., social anxiety[65]). In the sections below, we present preliminary data using measures of observable behavior (approach/avoidance conflict, facial expression coding, attentional bias), physiology (e.g., heart rate, skin conductance), and self-report (reward sensitivity, harm avoidance) to demonstrate that (1) we can reliably measure the constructs of interest and (2) empirically derive two dimensions that reflect positive and negative valence domains using data reduction methods.

Summary

The RDOC has identified positive and negative valence systems as constructs that can be used to relate altered neural systems functioning to anxiety and depression. Reward sensitivity, measured via reward-related decision-making paradigms, is viewed as a marker of the positive valence system. Threat sensitivity, measured via fear conditioning paradigms, is viewed as a marker of the negative valence system. The neural underpinnings of reward-related decision-making and fear conditioning are well understood. However, no studies to date have assessed the neural processes of both reward sensitivity and threat sensitivity as units of analysis among a larger set of measures (including cognitive, behavioral and physiological), within samples of anxious or depressed patients that is sufficiently large to generate a robust and reliable dimensional set of variables that quantify the positive and negative valence domains. That is the goal of this proposal. We hypothesize that measures of reward sensitivity, attentional bias towards positively valenced stimuli, approach behavior in an approach/avoidance task, propensity to display a happy facial expression while viewing positive stimuli, increased respiratory sinus arrhythmia (RSA; i.e., vagal tone) and activation in the ventral striatum during a reward processing task will load together on one latent construct (i.e., positive valence). Also, we hypothesize that measures of harm avoidance, attentional bias towards negatively valenced stimuli, avoidance behavior in a approach/avoidance task, propensity to display negative facial expressions (e.g., sadness, fear), increased heart rate, skin conductance, and EMG while viewing negative stimuli, and amygdala activation during a fear conditioning task will load together on a second latent construct (i.e., negative valence). Further, we hypothesize that latent factor scores of individuals with anxiety or depression will span this two-dimensional space. We aim to replicate these latent factor scores in a confirmatory sample, and test the stability of these scores in a test-retest assessment in a smaller subset of the larger sample. Our goal is to create a robust set of variables for measuring positive and negative valence that will become publically available for future research.

B. Innovation

The proposed project is innovative in the following ways:

(a) Extant research on the neural underpinnings of anxiety and depression has been largely constrained by comparing DSM-IV diagnosed subjects to healthy control subjects. Here, we propose a large sample of individuals seeking treatment for anxiety or depression, whose symptoms are such that they are impairing and driving treatment seeking, but may or may not meet criteria for a well-defined DSM-IV disorder. This approach is required to advance our understanding of the dimensions of psychopathology that are either shared across anxiety and depression (i.e., negative valence) or are more specific (although not exclusive) to depression (i.e., positive valence). Furthermore, whereas studies of the neural correlates of positive and negative valence systems are beginning to emerge, we are unaware of any studies that have included paradigms that are directly designed to assess both valence systems within the same sample. This will be the first time neural indices of positive valence and negative valence are investigated within the same broadly defined sample of anxious or depressed patients. Extant studies of neural processes in emotional disorders are limited to relatively small sample sizes. By combining resources across two sites, we will recruit a large enough sample to conduct more comprehensive analyses than ever before achieved.

(b) Although researchers have begun to evaluate relationships between specific neural processes, such as fear conditioning, and other “units of analysis” such as attentional bias to threat, no study to date attempts the comprehensive approach proposed herein for measuring multiple units of analysis for each valence system. That is, we propose to measure self-report, behavior, physiology, and neural circuits, and to assess their loading on constructs of positive and negative valence systems, and their relative contributions to each construct. We are employing an empirically derived approach, involving latent variable analyses, for developing a “battery of measures” for positive valence and negative valence, thus avoiding the tautology of relying upon a predetermined measure of each valence system against which to validate additional measures.

(c) The majority of the experimental approaches we propose for deriving variables (i.e., units of analysis) are well-established. However, we additionally include the automated facial action coding system (i.e., Computer Expression Recognition Toolbox; CERT) as a novel approach to measuring behavioral indices of affect on a millisecond scale in real time. Aside from its novelty and empirical grounding in a well-established literature of facial expression coding and emotion[66], a very attractive feature of CERT is its potential clinical utility. This measure can be easily incorporated into real world clinical settings, and provide the clinician with an objective index of positive or negative valence, that in turn eventually may guide treatment choice.

(d) We are collaborating with a statistician who has developed sophisticated analytical approaches to arrive at individual level outcome predictions (see letter of support from Dr. Wes Thompson). Specifically, we propose a three step approach consisting of (1) data reduction based on principal component analysis, (2) hypothesis-driven examination of the positive and negative valence domains using confirmatory factor analysis, and (3) latent variable analysis to examine the relationship between different units of analysis and underlying brain function to arrive at a comprehensive, robust, and reliable set of measures for quantifying negative and positive valence systems in clinically relevant populations.

(e) We aim to have robust and reliable dimensional measures that quantify the positive and negative valence systems. We will create a web-based repository to make these latent constructs (i.e. the measures and the derived factors), available to other researchers. This will promote the validation of the positive and negative valence systems in other clinical populations, and thus are truly generic domains as envisioned by the RDoC initiative. Finally, these latent constructs will serve as the platform for a subsequent proposal focused on treatment application; in other words, to what extent do treatments change measures of each construct, and to what extent do scores on each construct predict or moderate treatment outcomes?

C. Approach

Sample

We considered various populations based on our prior studies. We have conducted extensive studies in college populations[67-69], non-treatment seeking[70], and treatment seeking[71] psychiatric populations presenting to an Anxiety Disorders Clinic. We opted to identify individuals with anxiety or depression in a primary care setting, which we have used successfully to recruit a significant number of subjects[72], to maximize generalizability and external validity. Medication status was another important consideration. On the one hand, including medicated individuals with the possibility of using statistical techniques to account for their effect would provide a larger subject pool and could be conceived as being more representative. On the other hand, given our prior studies showing that antidepressants affect brain activation[73-75], a medication-free group would provide a more scientifically compelling approach to delineate the underlying latent constructs reflecting the positive and negative valence systems. Moreover, the former would require a much larger number of subjects and could not rule out a contribution of medication we chose the latter approach as detailed below.

Study clinics and subjects. We will recruit two waves of n=100 individuals each from two clinics (see power analysis): UCSD Primary Care Clinic and the UCLA Family Health Center (n=50 per wave per clinic). The UCLA clinic has 10 faculty physicians and 36 residents, the UCSD clinic is staffed by 40 faculty physicians. Consistent with our prior work[72], we expect to recruit a sample where ethnic minorities (approximately 40% in the UCLA clinic and 47% at the UCSD clinic) and low-income individuals (approximately 25% at both clinics) are well represented. Assuming equal non-refusal rates across sociodemographic groups, we project a final sample that is 50% female; 86% Caucasian, 6% African-American, 20% percent Hispanic, 6% Asian and 2% other; 25% low-income; and between 1-5% uninsured.

Facilitated Referral. Using methods we successfully used in the CALM study[72], subjects will be enrolled in this study through an approach we call “facilitated referral.” First, we will allow providers to refer potential subjects directly to our intake evaluation. Second, providers may screen patients in participating clinics using screening instruments described below, and if eligible, then offer the potential subject referral to our intake evaluation. This “facilitated screening and referral” is intended to capture subjects who might have anxiety or depression that are not known to the provider. To optimize recruitment and minimize attrition we have added a liaison at each clinic site who will aid in recruitment and enrollment

Subject inclusion and exclusion criteria. To enhance generalizability, and importantly, to enable selection of subjects in keeping with the RDoC intent, this study will feature broad inclusion criteria and narrow exclusion criteria. This approach was selected to maximize the clinical diversity of the study population. General **Inclusion criteria are:** (1) Referred for treatment of anxiety and/or depressive symptoms; (2) Score on the PHQ-9 is 10 or higher[76] and/or score on the OASIS is 8 or higher[77]; (3) Between the ages of 18-55; (4) Able to provide informed, written consent. Notably, in our prior CALM study, inclusion based on the OASIS plus a DSM-IV anxiety disorder yielded a highly comorbid anxious and depressed sample approximately 76% had comorbid depression). Thus, we fully expect that inclusion based on elevated anxiety (OASIS) or depression (PHQ-9) scores will yield a sample with the full range of anxiety and depression symptoms and associated functional impairment. **Exclusion criteria include:** The first 7 criteria below closely match procedures we successfully employed in our prior primary care CALM study (1) No telephone or easy access to telephone; (2) Alcohol or marijuana dependence (but not abuse) in the past year; (3) All other drug abuse or dependence in the past year. Subjects with alcohol or marijuana abuse in the past 12 months will be permitted in the study, but also will be given referrals for additional substance abuse treatment which may occur concurrently; (4) Bipolar I or Psychotic disorders; (5) Moderate to severe traumatic brain injury with evidence of neurological deficits, neurological disorders, or severe or unstable medical conditions that might be compromised by participation in the study (to be determined by primary care provider); (6) Active suicidal ideation; (7) Inability to

speak English; (8) Current use of a medication that could affect brain functioning, e.g., anxiolytics, antipsychotics, antidepressants, or mood stabilizers; (9) MRI exclusions: cardiac pacemaker, metal fragments in eyes/skin/body (shrapnel), aortic/aneurysm clips, prosthesis, by-pass surgery/coronary artery clips, hearing aid, heart valve replacement, shunt (ventricular or spinal), electrodes, metal plates/pins/screws/wires, or neuro/bio-stimulators (TENS unit), persons who have ever been a metal worker/welder, history of eye surgery/eyes washed out because of metal, vision problems uncorrectable with lenses, inability to lie still on one's back for 60 minutes; prior neurosurgery; tattoos with metal dyes, unwillingness to remove body piercings; (10) non-correctable vision or hearing problems, or color blindness, as some cognitive tests require intact sensory functioning. Patients who are excluded from the study will be referred outside of the study for additional care.

Further diagnostic assessment. Although, as noted above, entry into the study is not based on meeting diagnostic criteria for a particular mood or anxiety disorder, it will be important to characterize how our findings map onto DSM (currently DSM-IV, but we will adapt to using DSM-5 criteria, which will become available for the start date of this study). Accordingly, patients will complete a diagnostic interview with study personnel, using an abbreviated version of the Mini International Neuropsychiatric Interview (MINI Version 5.0.0 or its DSM-5 successor, when available)[78]. The MINI was chosen over other diagnostic interviews (e.g., SCID or CIDI) because of its relative brevity, good inter-rater reliability, and suitability for use by an interviewer with limited training, and because we used it in our previous primary care treatment study, CALM[72], and so we have extensive experience in its training, quality control, use and interpretation. We will include sections on PD, SAD, Posttraumatic Stress Disorder, GAD, OCD, and Major Depression and several modules to provide further clinical information or to determine ineligibility (Suicidality, Manic/Hypomanic Episode, Alcohol Abuse/Dependence, Drug Abuse/Dependence, and Psychotic Disorders).

Procedures: Patients presenting to the UCSD and UCLA clinics and completed complete the screening measures (OASIS and PHQ-9) are eligible subjects and will be asked to participate via our facilitated referral system. Individuals who accept an invitation to take part in the study will undergo a MINI diagnostic interview. Subjects included in the study will return for a behavioral testing session and a neuroimaging testing session. During the behavioral session participants will complete the self-report assessments, modified probe detection task, approach/avoidance conflict task, and emotional reactivity assessment in which they will view blocks of emotional images while state affect and physiology will be assessed throughout the procedures. The fMRI session will be scheduled within 2 days of the behavioral session and will comprise the following sequence of events: Prior to scanning, each subject will complete (1) informed consent, (2), self-report emotion assessments, (3) review of fMRI procedures and MRI safety screen, (4) review of instructions to complete the fear conditioning and reward tasks, and completion of practice tasks to ensure comprehension of instructions. The scanning protocol will consist of (1) reward processing task, (2) high-resolution structural scan, (3) fear conditioning task. At subset of participants (n=25 from each site) will be invited to return approximately one-month later to complete the same battery of assessments to establish test-retest reliability of the latent constructs (see Specific Aim 3).

Self-report Measures

(1) Positive and Negative Affective Schedule - State/Trait **(PANAS[79]):** The PANAS is a widely used measure comprising 20-items assessing activated forms of PA and NA using 5-point scales (1 = very slightly/not at all, 5 = extremely). To assess trait PA and NA, participants will be asked to respond according to how they have felt "during the past week". State PA and NA will be asked by asking participants to rate how they feel "right now (that is, at the present moment)" throughout the laboratory assessments following blocks of viewing positive, neutral, and negative IAPs images (see Emotional Reactivity section below). The PANAS has high internal consistency and temporal stability (trait version). Correlational data support its convergent and discriminant validity. Confirmatory factor analyses support the construct validity of the PANAS[80]. **(2) *Mood and Anxiety Symptom Questionnaire - Short Form*** **(MASQ[9]):** The MASQ is a 62-item measure of mood and anxiety symptoms developed to evaluate predictions of the tripartite model of anxiety and depression. The MASQ has four subscales: (1) General Distress Anxious Symptoms (GDA: 11 items), (2) General Distress Depressive Symptoms (GDD: 12 items), (3) Anxious Arousal (AA: 17 items), and (4) Anhedonic Depression (AD: 22 items). Respondents indicate the extent to which they experienced each symptom during the past week from 1=not at all to 5=extremely. The MASQ subscales have been found to have adequate convergent and discriminant validity as well as good internal consistency[81,82] in student, adult volunteer, and clinical samples. **(3) *Behavioral Inhibition and Activation Scales*** **(BIS/BAS[83]):** The behavioral inhibition and activation scales (BIS/BAS) include 20-items assessing dispositional BIS and BAS sensitivities (i.e. avoidance and approach motives), which are hypothesized to reflect the negative and positive valence systems, respectively. Items are rated on four-point scales (1 = strongly disagree; 4 = strongly agree). The BAS has three subscales (Drive, Reward Responsiveness, and Fun Seeking); however, factor analyses support a single higher-order factor. The BIS/BAS has good test-retest reliability. Correlational data support the relative orthogonality and convergent, discriminant, and predictive validity of the subscales[83].

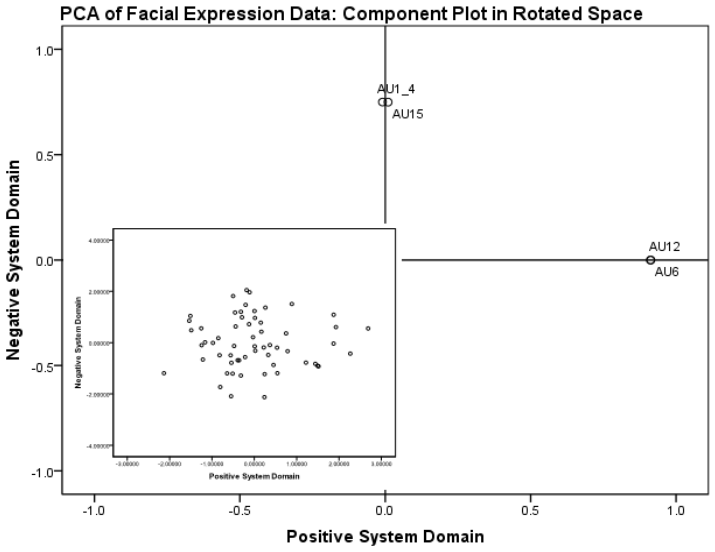
Behavioral Measures

***(1) Approach/Avoidance Conflict Task*[84]:** Participants will complete a computer-based approach-avoidance conflict (ACC) task. For each trial of the AAC, participants are shown a runway with pictures on each side to represent two potential outcomes. Each potential outcome includes an affective stimulus and a certain level of reward points. The valence of the affective stimulus is represented via a sun indicating a positive affective stimulus or a cloud indicating a negative affective stimulus. Level of reward points is represented by the red in the rectangles (more red = more points). The subject is to move an avatar on the runway (by pressing keys on a keyboard) to indicate their relative preference for each potential outcome. The location of the avatar at the end of the decision phase corresponds to the probability of each of the two outcomes occurring. If the subject moves the avatar to the middle of the runway, there is a 50% chance of each outcome; if he moves all the way to one side, there is a 90% chance of the nearest outcome and a 10% chance of the further outcome; and so on. Subjects therefore control the likelihood of the two outcomes but are unable to determine one or the other outcome for certain. The initial starting position of the avatar (at each of the nine possible positions, ranging from −4 to +4) is counterbalanced to enable characterization regarding the influence of effort on behavior. The affective stimuli used in the AAC paradigm include image and sound combinations (International Affective Picture System (IAPS[85]); International Affective Digitized Sounds (IADS[86]) and other freely available audio files). The "reward" includes 0, 2, 4, or 6 points presented along with a trumpet sound. There are three types of trials in the AAC task: (1) 'Avoidance-only', in which 0 points are offered for both outcomes. For these conditions, there is no explicit motivation to approach the negative affective outcome. (2) 'Approach-only', in which reward outcomes can be acquired in the absence of punishment, and hence there is no explicit motivation to avoid the reward outcome. (3) Three levels of 'Conflict' in which 2, 4, or 6 points are offered for the outcome involving a negative affective stimulus while 0 points are offered for the outcome involving a positive affective stimulus. These conditions are designed to produce approach-avoidance conflict, as the same behavior is associated both with reward and punishment. The main dependent measure will be the response latency from initial button press to end decision point, averaged across trials.

Preliminary evidence for Approach/Avoidance Conflict as a Behavioral Marker of Positive and Negative Valence Domains: *Background:* Research in animals and humans suggests that approach/avoidance conflict is a basic phenotype that reflects the relative strength of reward-related vs. aversive behavioral tendencies. Our group has developed a paradigm in which approach and avoidance drives can be measured behaviorally by manipulating the relative degree of reward and punishment associated with a given decision[84]. We examined whether approach vs. avoidance behavioral tendencies, as measured through an individual's response latency to approach vs. avoid rewarding or aversive outcomes, reflects distinct positive and negative dimensions. *Methods:* A cross-section of young adults recruited from UCSD/SDSU (N=184) completed a computerized approach-avoidance conflict (AAC) paradigm (described above). Response latency data were used to compute separate approach and avoidance bias scores for each level of conflict, yielding a total of 3 approach bias scores and 3 avoidance bias scores. *Results:* A principal component analysis with Promax rotation indicated that the data were best described by a 2-factor solution. The first dimension accounted for 56.1% of the variance and comprised the three avoidance conflict indices (negative valence domain). The second factor accounted for 25.0% of the variance and comprised the three approach conflict indices (positive valence domain). The two components were moderately related, $r(184) = .38$, but sufficiently distinct dimensions. *Conclusion:* Approach/avoidance conflict paradigms provide relatively independent indices of behavioral tendencies to approach rewarding outcomes vs. avoiding aversive outcomes, which may reflect positive vs. negative valence domains, respectively.

***(2) Facial Expressions*:** Advances in computer vision and machine learning over the past 15 years have led to the emergence of technology for automatic analysis of affective behavior[87]. During this time, the Machine Perception Laboratory at UCSD (MPLab) has focused on development of systems for automatic analysis of facial behavior, including audio-visual speech recognition[88-90] and recognition of facial expressions[89-93]. The output of the face detector is scaled to 90x90 and fed directly to the facial expression analysis system. First the face image is passed through a bank of Gabor filters at 8 orientations and 9 scales (2-32 pixels/cycle at 0.5 octave steps). The filterbank representations are then channeled to a classifier to code the image in terms of a set of expression dimensions. Research at the MPLab has demonstrated that performing feature selection on the Gabor filters prior to classification enhances both speed and accuracy. This approach combines feature selection based on Adaboost with feature integration using support vector machine. *Automatic Facial Expression Analysis*: A video camera will record each participant during viewing IAPs pictures (see Emotional Reactivity section below) in order to permit coding of facial expressions. Automatic facial expression analysis will be conducted by the Computer Expression Recognition Toolbox (CERT[94]), software developed and validated by our collaborators at the Machine Perception Laboratory at UCSD (MPLab). CERT analysis corresponds to the well-validated Facial Action Coding System (FACS[95,96]), a comprehensive method to objectively code facial expressions. CERT automatically codes the intensity of 26 component facial movements referred to as action units (AUs).

Preliminary evidence for Automatic Coding of Facial Expressions during Emotion Elicitation of Distinct Behavioral Indices of Positive and Negative Valence Domains: *Background:* Facial expressions are well-established as behavioral indices that reflect a person's emotions and internal states. The Facial Action Coding System (FACS[66]) is a reliable, valid, and widely used method to objectively code facial expressions. Previous studies suggest that particular component facial movements referred to as action units (AUs) reflect positive vs. negative emotional states[97]. For example, AU 12 (movement of the zygomatic major muscle) is associated with positive affective states whereas AU 15 ("lip corner depressors"; sadness) and AUs 1+4 (anxiety) are associated with negative affective states. To provide proof of concept, we used the Computer Expression Recognition Toolbox (CERT[89,94]) to automatically code facial expressions of participants completing impromptu videotaped speeches[98]. *Methods:* Videotaped speeches were obtained from N=59 participants from[98] recruited for a study of public speaking fears. We used CERT to automatically code the intensity of 26 AUs from FACs. Drawing on prior research[66,99], we examined the mean intensity rating for the following AUs averaged across the duration of the speech task: AUs 12 and 6 (positive emotion); AU 15 (sadness); AU 1+4 (anxiety). *Results:* A principal component analysis (PCA) with Promax rotation indicated that the data were best described by a 2-factor solution. The first dimension accounted for 41.6% of the variance and comprised AU 12 and 6 (positive valence domain). The second factor accounted for 28.1% of the variance and comprised AU 15 (sadness) and AU 1+4 (anxiety) (negative valence domain). The two components were orthogonal, $r(59) = .004$. See Figure for component plot (main figure) and scatterplot (inset) illustrating individual variability and independence of dimensions. *Conclusion:* Automatic coding of facial expressions may provide a validated and efficient method to measure independent dimensions of positive and negative behavioral domains.



(3) Modified Probe Detection Task: Attentional bias for positive and negative information will be measured using a version of the modified probe detection task (MPDT[100]). Emotional stimuli will comprise standardized male and female disgust, sad, happy, and neutral faces[101]. For emotional trials, each face will be paired with a neutral face from the same person (e.g., disgust–neutral). For baseline trials, a neutral face will be paired with a neutral face from the same person. Neutral-neutral trials serve as a baseline to compute facilitation and disengagement bias scores[102,103]. Participants will be presented with 128 trials—comprising all combinations of Actor (two male, two female), Face Pairs (happy-neutral, disgust-neutral, sad-neutral, neutral-happy, neutral-disgust, neutral-sad, neutral(h)-neutral(h), neutral(d)-neutral(d), neutral(s)-neutral(s)), Probe Type (E or F), Probe Location (Top or Bottom): 4 (Actor) x 8 (Face Pairs) x 2 (Probe Type) × 2 (Probe Location).

Evidence for the Independence of Attentional Bias Indices of Positive and Negative Valence Domains: *Background:* Attentional biases for emotional information are considered a hallmark of anxiety and depression[59,60]. Whereas selective processing of positive stimuli is associated with positive affective states[62], preferential attentional allocation for negative emotional information is associated with negative affective states (anxiety and depression). Evidence also suggests that aberrant attentional processing of positive information is associated with depression[64], and some anxiety disorders (e.g., social anxiety[65]). We adopted a dimensional approach to examine whether attentional biases for positive vs. negative stimuli reflect at least partially distinct dimensions. *Method:* A cross-section of healthy young adults (N = 194) recruited as part of prior studies[65] completed a well-established modified probe detection task (MPDT[100]) designed to measure attentional bias for positive and negative information (described above). Trials comprised negative-neutral, positive-neutral, or neutral-neutral face pairs. Neutral-neutral trials served as a baseline to compute facilitation and disengagement bias scores, yielding four attentional bias indices: (1) facilitation bias for positive; (2) facilitation bias for negative; (3) disengagement bias for positive; (4) disengagement bias for negative. *Results:* A principal component analysis with Promax rotation indicated that the data were best described by a 2-factor solution. The first dimension accounted for 41.9% of the variance and comprised the two negative bias indices (negative valence domain). The second factor accounted for 36.0% of the variance and comprised the two positive bias indices (positive valence domain). The two components were orthogonal, $r(194) = -.07$. *Conclusion:* Measures of attentional bias for positive and negative stimuli reflect distinct dimensions and therefore may serve as a cognitive marker of positive vs. negative valence domains, respectively.

Psychophysiological Measures: Research suggests that physiological responses to positive and negative imagery are moderated by trait affect. For example, Oliveira and colleagues[104] reported that individuals high in trait positive affect display attenuated autonomic reactions to distressing images when these images where preceded by a safety signal. Hofmann[105] reported that trait affect moderated the relationship between state affect and EEG responses during an imagery induction. In addition, individuals high in trait anger, enjoyment and surprise display inhibited startle responses to positive images[106]. Thus, given the well-established link between imagery and physiology[107] we will use the validated International Affective Picture System (IAPS[85]) to measure physiological responses to affective images.

(1) Emotional Reactivity*:** a series of 20 positive, 20 neutral and 20 negative slides (from the IAPS[85]) each presented for 5 sec in blocks of 10 with a 1 sec inter-trial interval within blocks and 120 sec interval between blocks. There will be two orders of trials to the block sequence. Order of slides will be fixed at first assessment and then counterbalanced as subsequent assessments. Images will be presented on a computer screen and interspersed with a fixation cross to which subjects are asked to attend. Heart rate (HR), respiratory sinus arrhythmia (RSA), skin conductance (SCR), and frontal electromyogram (EMG) will be recorded continuously using Biopac instrumentation (Lehigh, Pennsylvania). Data acquisition and storage will be facilitated by an IBM Pentium II, Keithley-Metrabyte A-D board, and Labview software. Raw data are continuously monitored via an oscilloscope. Data are sampled at a rate of 20Hz, and averaged over 30 second intervals corresponding to experimental periods. ***Heart Rate: Heart rate is recorded using standard limb electrocardiogram leads connected to a BIOPAC bioamplifier (S75-01), that inputs to a tachometer (S77-26) to plot beats per minute. ***Vagal tone*** will be measured via respiratory sinus arrhythmia (RSA), a non-invasive measure of cardiac vagal control characterized by increases in heart rate with inspiration and decreases in heart rate with expiration[108]. RSA will be computed from the R-wave to R-wave interbeat interval series in the frequency range of spontaneous breathing (.12 Hz - .40 Hz)[109]. ***Skin conductance:*** Skin conductance responses (SCRs) will be recorded with two Ag/AgCl electrodes filled with isotonic electrolyte gel. The electrodes will be attached to the thenar and hypothenar eminences of the left hand. Throughout the entire experiment a constant voltage of 0.5 V will be applied across the electrodes that are connected to a BIOPAC SCR 100 C amplifier with a gain of 2 mS/V. SCRs will be recorded continually on AcqKnowledge373 at a sampling rate of 1000 Hz during all study phases. ***Startle Reflex Electromyogram*:** Electromyogram (EMG) responses will be measured from the orbicularis oculi using two electrodes (Ag/AgCl, 11 mm outer diameter, 4 mm inner diameter contact surface) filled with electrode gel and fitted with adhesive collars. One electrode will be placed directly underneath the left eye, and another one approximately 1 cm to the left below the outside corner of the left eye. EMG activity will be amplified with a Biopac EMG amplifier and sampled (1000 Hz) by a Biopac MP150 workstation, filtered online with a passband of 28–500 Hz, rectified, and then smoothed (five-sample boxcar filter). Responses will be recorded on AcqKnowledge373 at a sampling rate of 1000 Hz.

Preliminary Evidence for Distinct Physiological Correlates of Positive and Negative Valence Systems: *Background:* Positive and negative affect are well-established in their association with anxiety and depression. However, it is less clear how positive and negative affect relate to psychophysiological measures in clinical samples of anxiety or depression. As a first step toward addressing this issue, we examined psychophysiological responses obtained from a mixed anxiety disorder sample exposed to stress-provoking and relaxation-inducing experimental conditions. *Methods:* Participants (N = 74) were a treatment-seeking sample from UCLA with a primary diagnosis of an anxiety disorder. During a pre-treatment laboratory assessment, participants were evaluated using the Positive and Negative Affect Scale (PANAS) and psychophysiological measures (i.e., heart rate (HR) and heart rate variability (HRV)). PANAS was measured at the beginning of baseline and end of a hyperventilation task and meditative relaxation task, and psychophysiology was measured throughout the tasks. *Results:* During the 15-minute meditative relaxation task, PA was associated with, a) high-frequency heart rate variability (HF-HRV) throughout all 15 minutes of the task and, b) root mean square of the successive differences (RMSSD) during the last five minutes. NA was associated with HR during the last five minutes of relaxation. Furthermore, baseline NA was negatively correlated with HF-HRV and positively correlated with RMSSD throughout all 15 minutes of relaxation and positively correlated with low-frequency/high-frequency heart rate variability (LF/HF-HRV) at the first and last five minutes. *Conclusions:* This study demonstrates that we can measure physiological responses under different experimental conditions, and that positive and negative affect systems may be associated with differential markers of physiological reactivity. In the current proposal, we will extend this approach by measuring physiological responses during exposure to positive, neutral, and negative affective images.

Neuroimaging

***(1) Reward Processing Task*:** We will employ a slow event-related fMRI card-guessing paradigm[46,47,110] for three reasons: First, it is well validated and widely used for reward-related brain function. Second, it specifically examines neural processes associated with basic reward-processing which most closely addresses

our interest in reward sensitivity. Third, it allows separate examination of reward-related brain function during anticipation and receipt of reward and loss. Our group has already employed this task. Trials will be presented in a pseudorandom order with predetermined outcomes. During each 20-sec trial, participants have 4-secs to guess, via button press, whether the value of a visually presented card with a possible value of 1-9 is higher or lower than 5. After a choice is made, the trial type is presented visually for 6-secs indicating whether the trial is a reward-anticipation (upward arrow) or loss-anticipation (downward arrow) type. In Reward-Anticipation trials, participants can win money if their guess is correct with no-change in earnings if their guess is incorrect. In Loss-Anticipation trials, participants lose money if their guess is incorrect with no-change in earnings if their guess is correct. The anticipation-period is immediately followed by the outcome-period, where participants are presented with the "actual" numerical value of the card (500ms) and receive outcome feedback (additional 500ms): a green upward arrow for win (Reward-Outcome), a red downward arrow for loss (Loss-Outcome), or a yellow circle for no-change feedback (No-Change). A crosshair is then presented for 9-sec (inter-stimulus-interval, ISI). The outcome-period is defined as a 7-sec period, from when participants are presented with the actual numerical value of the card, and the baseline-period comprises the last three secs of the ISI. 24 trials will be presented in 1 run with a balanced number of trial types within runs. Participants will be told they will receive $1 for each win, lose 50 cents for each loss, and obtain no earnings change for no-change-outcomes. Outcome probabilities will, in fact, be fixed such that participants receive the same earnings, although participants are led to believe their performance would determine net monetary gain. The task is programmed in E-prime 2.1 (Psychology Software Tools, Inc., Pittsburgh, PA) using a Windows-based operating system. Stimuli will be presented in the MR scanner using a screen at the foot of the scanner bed via back-projection from a head-coil-mounted mirror or high-resolution magnet-compatible goggles (Resonance Technology, Inc). Button press responses will be collected using an MR-compatible button box and recorded in E-prime.

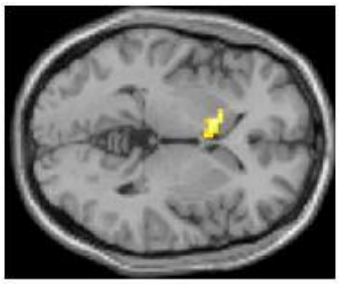


Figure 2. Striatal activation of combined UCLA/NWU Ps during anticipation of guaranteed rewards (-9, 9, -3, t=3.78, p<.05).

Preliminary evidence for ventral striatal activation during reward processing as part of the positive valence system: The UCLA site completed an fMRI pilot study of reward-related neural circuitry in healthy participants (n=7), using a task by Bar-Haim[111], wherein participants pressed one of two buttons to obtain a monetary reward. In guaranteed-reward trials, participants were told which button to press and got a reward for doing so; in non-guaranteed-reward trials, participants had to guess which button led to the reward (which was randomly determined). Neutral control trials had participants passively view geometric shapes. Two participants were scanned at another site (NWU) and five were scanned at UCLA, and all data were analyzed at UCLA. Initial analyses using a small-volume correction on a VS ROI showed significant activation in the striatum during anticipation of guaranteed rewards (-9, 9, -3, t=3.78, 20 voxels, see Figure 2), relative to neutral trials.

(2) Fear Conditioning Task: We will use a slow event-related fMRI differential Pavlovian fear-conditioning task, as in several prior fMRI studies of fear learning in healthy and anxious samples. All phases of conditioning (i.e., Habituation, Conditioning, Extinction, and Extinction Recall) will be completed during fMRI scanning. The US will consist of a shock applied to the fingers. The CS will consist of 3 different neutral facial expressions as human faces lead to stronger conditioning than geometric figures. One CS will be paired with the US during acquisition and subsequently extinguished (CS+E); one will be paired with the US during acquisition but never extinguished (CS+U); and one will never be paired with the US (CS-). The specific neutral facial expressions assigned to CS+E, CS+U, and CS- will be counterbalanced across participants.

Preliminary Evidence: The UCLA site completed a pilot study of an fMRI extinction recall paradigm in healthy Ps (n=10), aged 18-21. Participants (Ps) first completed habituation, fear conditioning, and extinction in the laboratory using two different neutral facial expressions as the CS+ and CS- and an uncomfortable scream as the US. Approximately two weeks later, Ps completed fMRI scanning while they were presented with both CSs again, in the form of morphed images that combined the CS+ and CS- faces in different percentages. Five Ps were scanned at another site (Northwestern University (NU) and five were scanned at UCLA. Data from NU were transferred to UCLA. All data were analyzed at UCLA and combined to examine group effects. Using parametric modulation analyses in which the stimulus-morphing range for each trial was entered as a regressor at the trial level, Ps exhibited significantly greater activity in subACC (3, 30, -12, t=3.20) in response to the CS+ vs. CS-

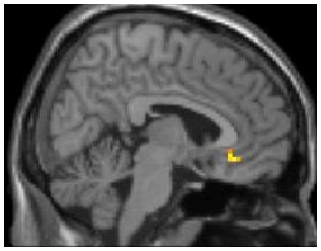


Figure 4. subACC activation of combined UCLA/NU Ps during extinction recall of CS+ vs CS- (3, 30, -12, t=3.20, p<.05).

(see Figure 1). Also, this subACC activation was negatively correlated with activity in the AMYG (-27, 0, -21, r=-.85, p<.05).

fMRI Data Processing

fMRI Image Acquisition: Neuroimaging data will be acquired using a Siemens Tim Trio 3 Tesla MRI scanners at the UCLA Staglin IMHRO Center for Cognitive Neuroscience and a GE Signa 3 Tesla MRI scanner at the Keck Center for Functional Neuroimaging at UCSD. Task parameters will be identical at both sites. There will be 3 functional runs (Habituation, Conditioning and Reward)in one session. Functional runs will utilize a gradient echo EPI sequence covering 39 axial slices (3.1mm thick; TR=2000ms; TE=25ms; FOV=200x200mm; Matrix=64x64; Flip Angle=90°). Structural 3D axial MPRAGE images will be acquired at both sessions to assist with image registration and normalization (1mm thick; TR=2200ms; TE=3.29ms; FOV=256x256; Matrix=256x256; Flip Angle=9°; 192 slices). Within fear learning, Habituation scan will last 4min12sec, and Conditioning will last 11min12sec. The Reward Processing Task will last 13min. To control random fluctuations in MRI field homogeneity and other factors (e.g., humidity and temperature) across the two scanning sessions, we will scan a standard phantom prior to each session and normalize data to the phantom for each P as necessary.

Cross-site imaging consistency: To maximize reliability of data collection across sites and the transfer of data from UCLA to UCSD: (1) Initial phantom scans for site qualification will be used; (2) UCLA and UCSD scans will be transferred to the neuroimaging data server at UCSD within 48 hours of acquisition and kept in a separate 'quarantine' area until put through a standard quality control pipeline to examine for artifacts, movement, etc. Scans that pass this quality assurance will be placed into the main database. UCLA data will be transferred to the UCSD server via a File Transfer Protocol (FTP) between the server at UCSD's Keck Center for Functional MRI (CFMRI), where UCSD data are collected, and the FUNC server at UCLA, (3) quality checks of data will be conducted and quality assurance scans conducted, and (4) analysis for site effects will be incorporated into the testing of primary study hypotheses. A variety of metrics have been used to assess inter-scanner reliability, such as volume of activation or BOLD signal change, and the automated quality assurance routine, which gives one dispersion measure per volume (averaged across voxels) expressed as percent signal change relative to the mean of the time-series. This measure will be incorporated in first-level analyses as a covariate of no interest to account for differences in data quality. Intraclass correlation coefficients (ICC) commonly based on a measure of effect size will be computed from this quality measure to estimate within and between site reliability. The data preprocessing and modeling pathway will also utilize field maps to correct for individual field in homogeneities of each scanner and several regressors of no interest will be included to account for reliability in between site variance (using realignment parameters, and the quality measure as regressors).

***fMRI Data Analysis. Image analysis pathway*:** The basic structural and functional image processing will be done with the Analysis of Functional Neuroimages (AFNI) software package[112]. A multivariate regressor approach detailed below will be used to relate changes in EPI intensity to differences in task characteristics. EPI images will be co-registered using a 3D-coregistration algorithm that has been developed to minimize the amount of image translation and rotation relative to all other images. Six motion parameters will be obtained across the time series for each subject. Based on the distribution of these average motion parameters, subjects will be excluded if the average in any one of these parameters exceeds 2 standard deviations from the mean or if movement exceeds the size of the voxel (4 mm). This assures that differences between groups are not due to differences in movements during scanning. Motion parameters will be used as regressors to adjust EPI intensity changes due to motion artifacts. This has been shown to increase power in detecting task-related activation. All slices of the EPI scans will be temporally aligned following registration to ensure different relationships with the regressors are not due to the acquisition of different slices at different times during the repetition interval.

Multiple regressor analyses: Regressors of interest have been described above for each task separately. Specifically, a 0-1 reference function will be convolved with a gamma variate function to model a prototypical hemodynamic response (6-8 second delay) and to account for the temporal dynamics of the hemodynamic response (typically 12-16 seconds)[113] . The convolved time series will be normalized and used as a regressor of interest. A series of regressors of interest and the motion regressors will be entered into the AFNI program 3dDeconvolve to determine the height of each regressor for each subject. The key measure is the voxel-wise normalized relative signal change (or % signal change for short), which is obtained by dividing the regressor coefficient by the sum of the zero-order regressor and the mean first-order regressor. In previous studies, SPATIAL smoothing using a filter with full width half maximum of approximately 1-2 voxel-size (i.e., 3-6 mm) has resulted in intra-group variability of EPI scans that parallel those observed for averages of the structural MR images and has been reported in the literature to yield the highest detection power[114]. Spatially smoothed % signal change data will be transformed into Talairach coordinates based on the anatomical MR image, which is transformed manually in AFNI.

***Second level analyses*:** The voxel-wise Talairach-transformed % signal change data is the main dependent measure. For fear learning, contrasts include CS+E + CS+U vs. CS- to assess activity during Conditioning. For reward, contrasts of interest include Reward-Anticipation vs. baseline and Reward-Outcome vs. baseline, indexing the expectation and receipt of reward, respectively. Similar contrasts will be used for Loss-Anticipation and Loss-Outcome. Movement parameters from the realignment stage will be entered as covariates of no interest in these analyses to control for participant movement. The dependent measures will be entered into a *mixed effects model*. Mixed effects models offer a flexible framework to model the sources of variation and correlation[115]. In particular, correlation among the data occurs when a number of observations are taken on the same observational unit over time. In addition mixed effects models are particularly robust for the analysis of unbalanced data when compared to similar analyses within the general linear model framework. We will use the implementation of the linear mixed effects models in R[116], which estimates the parameters of the mixed model using Maximum Likelihood Estimation (MLE) or Restricted Maximum Likelihood Estimation (RMLE) procedure. These calculations will be done within the R computing environment using routines that read in AFNI data sets.

General Statistical Approach: The goal of this proposal is to derive latent variables that adequately quantify the positive and negative valence domain across different units of analyses. The analysis of the variables that are extracted from each unit (as shown in Figure below) will consist of three steps. First, a principal component analysis will be conducted for each unit of analysis to determine the number of independent degrees of freedom contributing to the variance observed in each unit. For example, we will utilize all facial action units, which have been recorded during viewing of IAPS pictures in a principal components analysis. We expect to extract at least two independent components (as shown in the pilot data sections above). The action units that show the highest correlation with the components will be used for subsequent analyses. Second, we will conduct a confirmatory factor analysis with the variables from each unit of analysis that showed the highest correlation with the principal components and propose two factors – positive valence system and negative valence system. We will subsequently test the statistical significance of the coefficients contributing to the factors. Finally, we will conduct a latent variable analysis as detailed below to relate one unit directly to another unit of analysis.

Unit of Analysis	Measure	Positive System Domain	Negative System Domain
Neural/Circuits	Reward paradigm: BOLD % signal difference during reward or loss trials in anatomically constrained ROIs for (1) Anticipation & (2) Outcome phase	Reward trials - anticipation & outcome phase: Ventral striatum, dorsal striatum (caudate), OFC, amygdala	Loss trials - anticipation & outcome phase: Ventral striatum, dorsal striatum (caudate), OFC, amygdala
	Fear conditioning task: BOLD % signal difference in anatomically constrained ROIs for Conditioning trials (CS+E + CS+U vs. CS-)	n/a	Amygdala, insula
Physiology	HR, SCR, EMG, & RSA while viewing emotional images (IAPs)	RSA/vagal tone - resting state; viewing positive images	HR, SCR, EMG - viewing negative images
Behavior: Facial Expressions	Automatic Facial Action Coding System while viewing emotional images (IAPs)	Positive facial expressions (e.g., AUs 12, 6)	Negative facial expressions (e.g., AUs 4, 15, 1+4, 23, 24)
Behavior: Approach/avoidance conflict	Approach/avoidance conflict task	Response latencies to approach reward outcomes on conflict trials relative to approach only (non-conflict) trials	Response latencies to avoid punishment outcomes on conflict trials relative to avoid only (non-conflict) trials
Behavior: Attentional bias	Probe detection task (Positive, negative, neutral stimuli)	Response latencies to identify probe appearing in the vicinity of positive relative to neutral stimuli	Response latencies to identify probe appearing in the vicinity of negative relative to neutral stimuli
Self-report: General emotion/personality	PANAS-Trait; PANAS-State (viewing emotional images, IAPs); MASQ; BIS/BAS	Positive Affect: Trait; State - viewing positive stimuli; Anhedonic Depression; BAS	Negative Affect: Trait; State - viewing aversive stimuli; General Distress; Anxious arousal; BIS
Self-report related to diagnostic constructs	OASIS (anxiety); PHQ-9 (depression); SDS & QOLI (functional interference)	PHQ-9, SDS, QOLI	OASIS, PHQ-9, SDS, QOLI

Power and sample size considerations: Currently there is no estimation for sample size for factor analysis that is based on statistical theory. The traditional perspective is that there should be at least 10 cases for each item in the instrument being used[117], which has been found to often yield inadequate results[118]. However, one can use several approaches to determine whether the sample size is adequate for the proposed study. First, sample size depends on the ratio of the number of variables to the number of factors, and the proportion of the total variance in the data that is extracted by the factor analysis[119]. With 10 variables and 2

proposed factors even given a high common variance a sample size of 50 subjects should suffice. Alternatively, one can take a multiple regression approach to approximate the variance that needs to be accounted for by a given variable. For example, given a sample size of n=100, and 10 explanatory variables in the CFA, the minimum detectable $r^2 = 0.156$ with an alpha of 0.05 and a beta of 1–0.8[120]. In other words, our approach will be successful if the measurement captures at least 15% of the variance observed in the population. Our prior single regressor analyses revealed correlation coefficients of 0.4 and above, which translates to explaining at least 16% and is therefore consistent with our proposed sample size. Moreover, according to Westland[118], several considerations should be given when examining sample sizes for structural equation models. Among these is the notion that latent variables impose an a-priori belief system and re-attribute the variance found among the measures across different levels of analyses. Given our sample size of n=100, we will be able to examine a ratio of indicators to latent variables of > 3. In other words, we will be able to examine at least three latent constructs at one time.

Linear latent variable approach: There is no uniformly agreed upon definition of latent variable analysis [121]. However, one approach is to define a latent variable as a variable for which there is no sample realization for at least some observations in a given sample. In this context, the positive or negative valence domain can be considered a latent variable. More formally, the latent variable model consists of a measurement part describing the responses $\mathbf{Y}i = (\mathbf{Y}i\mathbf{1}, \ldots, \mathbf{Y}ip)'$:

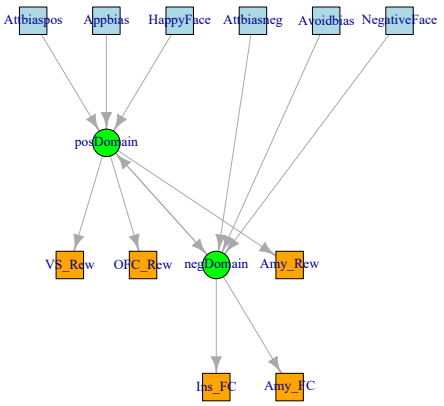
$$Y_{ij} = \nu_j + \sum_{k=1}^{l} \lambda_{jk}\eta_{ik} + \sum_{r=1}^{q} \kappa_{jr}X_{ir} + \sum_{k=1}^{l} \delta_{jk}V_{ijk}\eta_{ik} + \epsilon_{ij},$$

and a structural part describing the latent variables $\boldsymbol{\eta}i = (\boldsymbol{\eta}i\mathbf{1}, \ldots, \boldsymbol{\eta}il)'$: where i = 1, . . . , n is the index of the sampling unit (e.g. individuals), j = 1, . . . , p is the index of the observed variables (measurements or within cluster observations) and s = 1, . . . , l is the index of the l distinct latent variables. Fundamentally, latent variable approaches can be divided into posteriori latent variables, which are latent variables derived from the

$$\eta_{is} = \alpha_s + \sum_{k=1}^{l} \beta_{sk}\eta_{ik} + \sum_{r=1}^{q} \gamma_{sr}X_{ir} + \sum_{k=1}^{l} \tau_{sk}W_{isk}\eta_{ik} + \zeta_{is},$$

data analysis and a priori latent variables are hypothesized prior to an examination of the data. The approach proposed here is to utilize a-posteriori approaches with various latent variable models for the exploratory sample and to transition to the a-priori approach for the confirmatory sample. The goal of this proposal is to develop a heuristic framework that will enable one to combine the different units of analysis in a hypothesis-driven manner. Practically, this translates to establishing different structures that are superimposed on the measurement part and examining statistically whether certain structures provide better explanation of the observed variation than others.

***Implementation of the latent variable approach*:** We will use the implementation of the Linear Latent Variable model provided by the *lava*-package[122] as part of the R[116]. This package provides a very general modeling framework, which includes structural equation models and mixed models as important special cases. This model class also allows for non-linear effects of covariates and non-linear parameter constraints. The models are specified independently of data using commands that are similar to standard regression modeling in R. The lava-package also includes sophisticated inferential methods such as multi-group analyses, robust standard errors for clustered correlated data, maximum likelihood based inference with data missing at random and inference for indirect and total effects.



Path diagram of an example model with correlation between residuals of the latent variables. Observed variables are framed with rectangles whereas latent variables are framed with ellipses. Regression associations are depicted as one-headed arrows and covariance or correlations are shown as (dashed) double-headed arrows; color-codes: exogenous:=light blue, endogenous:=orange, latent:=green.

***Example of latent variable approach relating approach/avoidance task to activation of brain areas during reward paradigm and fear conditioning paradigm*:** This latent model was created with the lava package in R. We assumed that three units of analysis (attentional biases, facial expressions and approach/avoidance behavior) contribute differentially to positive and negative value systems (domains). These latent factors – in turn – determine the degree of activation in ventral striatum (VS) orbitofrontal cortex (OFC) and amygdala (AMY) during reward processing and insula (IN) and amygdala (AMY) during fear conditioning (FC). The regression coefficients can subsequently be used to calculate for each subject the degree of positive and negative valence system "state", which provides the measure that we termed the deliverable of this project.

Test/Re-test Reliability: We will utilize standard test/re-test approaches using the omega function implemented in the psych package that is available in R. This tool calculates standard ICCs but also Cronbach's coefficient and Guttman's estimates.

References

1. American Psychiatric A. *Diagnostic and Statistical Manual of Mental Disorders (4th Edition): DSM-IV*. Washington: The American Psychiatric Association; 1994.
2. Sanislow CA, Pine DS, Quinn KJ, et al. Developing constructs for psychopathology research: research domain criteria. *J Abnorm Psychol.* Nov 2010;119(4):631-639.
3. Insel T, Cuthbert B, Garvey M, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry.* Jul 2010;167(7):748-751.
4. Health NIoM. Positive Valence Systems: Workshop Proceedings. http://www.nimh.nih.gov/research-funding/rdoc/positive-valence-systems-workshop-proceedings.shtml. Accessed 10/12/2012, 2012.
5. Health NIoM. Negative Valence Systems: Workshop Proceedings. http://www.nimh.nih.gov/research-funding/rdoc/negative-valence-systems-workshop-proceedings.shtml. Accessed 10/12/2012, 2012.
6. Kessler RC, Berglund P, Demler O, et al. The Epidemiology of Major Depressive Disorder: Results From the National Comorbidity Survey Replication (NCS-R). *JAMA.* 2003;289(23):3095-3105.
7. Kessler RC, Ruscio AM, Shear K, Wittchen HU. Epidemiology of anxiety disorders. *Curr Top Behav Neurosci.* 2010;2:21-35.
8. James W. *The principles of psychology*. New York: H. Holt and Company; 1988.
9. Clark LA, Watson D. Tripartite model of anxiety and depression: psychometric evidence and taxonomic implications. *J Abnorm Psychol.* Aug 1991;100(3):316-336.
10. Chorpita BF. The tripartite model and dimensions of anxiety and depression: an examination of structure in a large school sample. *J Abnorm.Child Psychol.* 2002;30(2):177-190.
11. Chorpita BF, Albano AM, Barlow DH. The structure of negative emotions in a clinical sample of children and adolescents. *J Abnorm Psychol.* Feb 1998;107(1):74-85.
12. Weinstock LM, Whisman MA. Neuroticism as a common feature of the depressive and anxiety disorders: a test of the revised integrative hierarchical model in a national sample. *J Abnorm.Psychol.* 2006;115(1):68-74.
13. Craske MG, Rauch SL, Ursano R, Prenoveau J, Pine DS, Zinbarg RE. What is an anxiety disorder? *Depress Anxiety.* 2009;26(12):1066-1085.
14. O'Doherty JP, Deichmann R, Critchley HD, Dolan RJ. Neural Responses during Anticipation of a Primary Taste Reward. *Neuron.* 2002;33(5):815-826.
15. O'Doherty J, Rolls ET, Francis S, et al. Sensory-specific satiety-related olfactory activation of the human orbitofrontal cortex [corrected and republished in Neuroreport 2000 Mar 20;11(4):893-7]. *Neuroreport.* 2000;11(2):399-403.
16. O'Doherty J, Kringelbach ML, Hornak J, Andrews C, Rolls ET. Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat.Neurosci.* 2001;4(1):95-102.
17. Zink CF, Pagnoni G, Martin-Skurski ME, Chappelow JC, Berns GS. Human striatal responses to monetary reward depend on saliency. *Neuron.* 2004;42(3):509-517.
18. Delgado MR, Miller MM, Inati S, Phelps EA. An fMRI study of reward-related probability learning. *Neuroimage.* 2005;24(3):862-873.
19. Knutson B, Fong GW, Adams CM, Varner JL, Hommer D. Dissociation of reward anticipation and outcome with event-related fMRI. *Neuroreport.* 2001;12(17):3683-3687.
20. Samanez-Larkin GR, Gibbs SE, Khanna K, Nielsen L, Carstensen LL, Knutson B. Anticipation of monetary gain but not loss in healthy older adults. *Nat.Neurosci.* 2007;10(6):787-791.
21. Ernst M, Nelson EE, Jazbec S, et al. Amygdala and nucleus accumbens in responses to receipt and omission of gains in adults and adolescents. *Neuroimage.* 2005;25(4):1279-1291.
22. Kringelbach ML. The human orbitofrontal cortex: linking reward to hedonic experience. *Nat.Rev.Neurosci.* 2005;6(9):691-702.
23. De Martino F, Valente G, Staeren N, Ashburner J, Goebel R, Formisano E. Combining multivariate voxel selection and support vector machines for mapping and classification of fMRI spatial patterns. *Neuroimage.* Oct 15 2008;43(1):44-58.
24. Zalla T, Koechlin E, Pietrini P, et al. Differential amygdala responses to winning and losing: a functional magnetic resonance imaging study in humans. *Eur.J.Neurosci.* 2000;12(5):1764-1770.
25. Breiter HC, Aharon I, Kahneman D, Dale A, Shizgal P. Functional imaging of neural responses to expectancy and experience of monetary gains and losses. *Neuron.* 2001;30(2):619-639.
26. Baxter MG, Murray EA. The amygdala and reward. *Nat.Rev.Neurosci.* 2002;3(7):563-573.
27. Bush G, Vogt BA, Holmes J, et al. Dorsal anterior cingulate cortex: A role in reward-based decision making. *Proc.Natl.Acad.Sci.U.S.A.* 2002;99(1):523-528.

28. Berns GS, McClure SM, Pagnoni G, Montague PR. Predictability modulates human brain response to reward. *J Neurosci.* 2001;21(8):2793-2798.
29. Pizzagalli DA, Iosifescu D, Hallett LA, Ratner KG, Fava M. Reduced hedonic capacity in major depressive disorder: evidence from a probabilistic reward task. *J Psychiatr Res.* Nov 2008;43(1):76-87.
30. Pizzagalli DA, Holmes AJ, Dillon DG, et al. Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *Am J Psychiatry.* Jun 2009;166(6):702-710.
31. Davidson RJ. Affective style, psychopathology, and resilience: brain mechanisms and plasticity. *Am.Psychol.* 2000;55(11):1196-1214.
32. Der-Avakian A, Markou A. The neurobiology of anhedonia and other reward-related deficits. *Trends Neurosci.* Jan 2012;35(1):68-77.
33. Treadway MT, Zald DH. Reconsidering anhedonia in depression: lessons from translational neuroscience. *Neurosci Biobehav Rev.* Jan 2011;35(3):537-555.
34. Eshel N, Roiser JP. Reward and punishment processing in depression. *Biol Psychiatry.* Jul 15 2010;68(2):118-124.
35. Elman I, Lowen S, Frederick BB, Chi W, Becerra L, Pitman RK. Functional neuroimaging of reward circuitry responsivity to monetary gains and losses in posttraumatic stress disorder. *Biol Psychiatry.* Dec 15 2009;66(12):1083-1090.
36. Sailer U, Robinson S, Fischmeister FP, et al. Altered reward processing in the nucleus accumbens and mesial prefrontal cortex of patients with posttraumatic stress disorder. *Neuropsychologia.* Sep 2008;46(11):2836-2844.
37. Guyer AE, Choate VR, Detloff A, et al. Striatal functional alteration during incentive anticipation in pediatric anxiety disorders. *Am J Psychiatry.* Feb 2012;169(2):205-212.
38. Bouton ME, King DA. Contextual control of the extinction of conditioned fear: tests for the associative value of the context. *J.Exp.Psychol.Anim.Behav.Process.* 1983;9(3):248-265.
39. Griez E. Experimental models of anxiety. Problems and perspectives. *Acta Psychiatr Belg.* 1984;84:511-532.
40. Davis M. Pharmacological and anatomical analysis of fear conditioning using the fear-potentiated startle paradigm. *Behav.Neurosci.* 1986;100(6):814-824.
41. Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav.Neurosci.* 1992;106(2):274-285.
42. Labar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron.* 1998;20(5):937-945.
43. Buchel C, Dolan RJ. Classical fear conditioning in functional neuroimaging. *Curr.Opin.Neurobiol.* 2000;10(2):219-223.
44. Delgado MR, Olsson A, Phelps EA. Extending animal models of fear conditioning to humans. *Biol.Psychol.* 2006;73(1):39-48.
45. Etkin A, Wager TD. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry.* Oct 2007;164(10):1476-1488.
46. Delgado MR, Locke HM, Stenger VA, Fiez JA. Dorsal striatum responses to reward and punishment: effects of valence and magnitude manipulations. *Cogn Affect Behav Neurosci.* Mar 2003;3(1):27-38.
47. Delgado MR, Nystrom LE, Fissell C, Noll DC, Fiez JA. Tracking the hemodynamic responses to reward and punishment in the striatum. *J Neurophysiol.* Dec 2000;84(6):3072-3077.
48. Knutson B, Bhanji JP, Cooney RE, Atlas LY, Gotlib IH. Neural responses to monetary incentives in major depression. *Biol Psychiatry.* Apr 1 2008;63(7):686-692.
49. Phelps EA, Delgado MR, Nearing KI, LeDoux JE. Extinction learning in humans: role of the amygdala and vmPFC. *Neuron.* Sep 16 2004;43(6):897-905.
50. Craske MG, Rose RD, Lang A, et al. Computer-assisted delivery of cognitive behavioral therapy for anxiety disorders in primary-care settings. *Depress Anxiety.* 2009;26(3):235-242.
51. Bremner JD. Effects of traumatic stress on brain structure and function: relevance to early responses to trauma. *J.Trauma Dissociation.* 2005;6(2):51-68.
52. Milad MR, Pitman RK, Ellis CB, et al. Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry.* Dec 15 2009;66(12):1075-1082.
53. Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biol.Psychiatry.* 2001;50(9):651-658.

54. Fusar-Poli P, Placentino A, Carletti F, et al. Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. *J Psychiatry Neurosci.* Nov 2009;34(6):418-432.
55. Hariri AR, Tessitore A, Mattay VS, Fera F, Weinberger DR. The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage.* 2002;17(1):317-323.
56. Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch.Gen.Psychiatry.* 2005;62(5):529-535.
57. Zalsman G, Mann JJ. The neurobiology of suicide in adolescents. An emerging field of research. *Int.J.Adolesc.Med.Health.* 2005;17(3):195-196.
58. Stoy M, Schlagenhauf F, Sterzer P, et al. Hyporeactivity of ventral striatum towards incentive stimuli in unmedicated depressed patients normalizes after treatment with escitalopram. *J Psychopharmacol.* May 2012;26(5):677-688.
59. Taylor CT, Amir N. *Attention and emotion regulation*. New York: Guilford Press; 2010.
60. Bar-Haim Y, Lamy D, Pergamin L, Bakermans-Kranenburg MJ, van IMH. Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychol Bull.* Jan 2007;133(1):1-24.
61. El Khoury-Malhame M, Reynaud E, Soriano A, et al. Amygdala activity correlates with attentional bias in PTSD. *Neuropsychologia.* Jun 2011;49(7):1969-1973.
62. Tamir M, Robinson MD. The happy spotlight: positive mood and selective attention to rewarding information. *Pers Soc Psychol Bull.* Aug 2007;33(8):1124-1136.
63. Gable PA, Harmon-Jones E. Late positive potential to appetitive stimuli and local attentional bias. *Emotion.* Jun 2010;10(3):441-446.
64. Joormann J, Talbot L, Gotlib IH. Biased processing of emotional information in girls at risk for depression. *J Abnorm Psychol.* Feb 2007;116(1):135-143.
65. Taylor CT, Bomyea J, Amir N. Attentional bias away from positive social information mediates the link between social anxiety and anxiety vulnerability to a social stressor. *J Anxiety Disord.* May 2010;24(4):403-408.
66. Ekman P, Friesen WV. *Facial Action Coding System: A Technique for the Measurement of Facial Movement*. Palo Alto: Consulting Psychologists Press; 1978.
67. Paulus MP, Feinstein JS, Simmons A, Stein MB. Anterior cingulate activation in high trait anxious subjects is related to altered error processing during decision making. *Biol.Psychiatry.* 2004;55(12):1179-1187.
68. Stein MB, Simmons AN, Feinstein JS, Paulus MP. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *Am.J.Psychiatry.* 2007;164(2):318-327.
69. Simmons A, Matthews SC, Feinstein JS, Hitchcock C, Paulus MP, Stein MB. Anxiety vulnerability is associated with altered anterior cingulate response to an affective appraisal task. *Neuroreport.* 2008;19(10):1033-1037.
70. Stein MB, Kennedy CM, Twamley EW. Neuropsychological function in female victims of intimate partner violence with and without posttraumatic stress disorder. *Biol.Psychiatry.* 2002;52(11):1079-1088.
71. Aupperle RL, Allard CB, Grimes EM, et al. Dorsolateral prefrontal cortex activation during emotional anticipation and neuropsychological performance in posttraumatic stress disorder. *Arch Gen Psychiatry.* Apr 2012;69(4):360-371.
72. Roy-Byrne P, Craske MG, Sullivan G, et al. Delivery of evidence-based treatment for multiple anxiety disorders in primary care: a randomized controlled trial. *JAMA : the journal of the American Medical Association.* May 19 2010;303(19):1921-1928.
73. Arce E, Simmons AN, Lovero KL, Stein MB, Paulus MP. Escitalopram effects on insula and amygdala BOLD activation during emotional processing. *Psychopharmacology (Berl).* 2008;196(4):661-672.
74. Simmons A, Strigo IA, Matthews SC, Paulus MP, Stein MB. Initial Evidence of a Failure to Activate Right Anterior Insula During Affective Set Shifting in Posttraumatic Stress Disorder. *Psychosom Med.* 2009;71(4):373-377.
75. Matthews SC, Simmons AN, Strigo IA, Arce E, Stein MB, Paulus MP. Escitalopram attenuates posterior cingulate activity during self-evaluation in healthy volunteers. *Psychiatry Res.* 2010;182(2):81-87.
76. Manea L, Gilbody S, McMillan D. Optimal cut-off score for diagnosing depression with the Patient Health Questionnaire (PHQ-9): a meta-analysis. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne.* Feb 21 2012;184(3):E191-196.

77. Campbell-Sills L, Norman SB, Craske MG, et al. Validation of a brief measure of anxiety-related severity and impairment: the Overall Anxiety Severity and Impairment Scale (OASIS). *Journal of affective disorders.* Jan 2009;112(1-3):92-101.
78. Sheehan DV, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry.* 1998;59 (suppl 20):22-33.
79. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol.* Jun 1988;54(6):1063-1070.
80. Crawford JR, Henry JD. The positive and negative affect schedule (PANAS): construct validity, measurement properties and normative data in a large non-clinical sample. *Br J Clin Psychol.* Sep 2004;43(Pt 3):245-265.
81. Watson D, Clark LA, Weber K, Assenheimer JS, Strauss ME, McCormick RA. Testing a tripartite model: II. Exploring the symptom structure of anxiety and depression in student, adult, and patient samples. *J Abnorm Psychol.* Feb 1995;104(1):15-25.
82. Watson D, Weber K, Assenheimer JS, Clark LA, Strauss ME, McCormick RA. Testing a tripartite model: I. Evaluating the convergent and discriminant validity of anxiety and depression symptom scales. *J Abnorm Psychol.* Feb 1995;104(1):3-14.
83. Carver CS, White TL. Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS scales. *Journal of Personality and Social Psychology.* 1994;67:319-333.
84. Aupperle RL, Sullivan S, Melrose AJ, Paulus MP, Stein MB. A reverse translational approach to quantify approach-avoidance conflict in humans. *Behav Brain Res.* Aug 6 2011.
85. Lang PJ, Bradley MM, Cuthbert BN. *International affective picture system (IAPS): Affective ratings of pictures and instruction manual. Technical Report A-8.* Gainesville, FL: The Center for Research in Psychophysiology, University of Florida; 2008.
86. Bradley MM, Lang PJ. *International affective digitized sounds (IADS): Stimuli, instruction manual and affective ratings (Tech. Rep. No. B-2).* Gainsville, FL: The Center for Research in Psychophysiology, University of Florida; 1999.
87. Pantic M, Rothkrantz LJ. Facial action recognition for facial expression analysis from static face images. *IEEE Trans Syst Man Cybern B Cybern.* Jun 2004;34(3):1449-1461.
88. Wu T, Butko NJ, Ruvolo P, Whitehill J, Bartlett MS, Movellan JR. Multilayer Architectures for Facial Action Unit Recognition. *IEEE Trans Syst Man Cybern B Cybern.* May 11 2012.
89. Susskind JM, Littlewort G, Bartlett MS, Movellan J, Anderson AK. Human and computer recognition of facial expressions of emotion. *Neuropsychologia.* Jan 7 2007;45(1):152-162.
90. Bartlett MS, Movellan JR, Sejnowski TJ. Face recognition by independent component analysis. *IEEE Trans Neural Netw.* 2002;13(6):1450-1464.
91. Donato G, Bartlett MS, Hager JC, Ekman P, Sejnowski TJ. Classifying Facial Actions. *IEEE Trans Pattern Anal Mach Intell.* Oct 1999;21(10):974.
92. Bartlett MS, Hager JC, Ekman P, Sejnowski TJ. Measuring facial expressions by computer image analysis. *Psychophysiology.* Mar 1999;36(2):253-263.
93. Bartlett MS, Sejnowski TJ. Learning viewpoint-invariant face representations from visual experience in an attractor network. *Network.* Aug 1998;9(3):399-417.
94. Littlewort G, Whitehill J, Wu T, et al. The Computer Expression Recognition Toolbox (CERT). Paper presented at: IEEE International Conference on Automatic & Gesture Recognition and Workshops, 2011.
95. Ekman P, Levenson RW, Friesen WV. Autonomic nervous system activity distinguishes among emotions. *Science.* 1983;221(4616):1208-1210.
96. Young AW, Rowland D, Calder AJ, Etcoff NL, Seth A, Perrett DI. Facial expression megamix: tests of dimensional and category accounts of emotion recognition. *Cognition.* 1997;63(3):271-313.
97. Ekman P. Facial Expression and Emotion. *American Psychologist.* 1993;48(4):384-392.
98. Taylor CT, Bomyea J, Amir N. Malleability of attentional bias for positive emotional information and anxiety vulnerability. *Emotion.* Feb 2011;11(1):127-138.
99. Ekman P, Friesen W, Ancoli S. Facial signs of emotional experience. *Journal of Personality and Social Psychology.* 1980;39(6):1125-1134.
100. MacLeod C, Mathews A. Anxiety and the allocation of attention to threat. *Q J Exp Psychol A.* Nov 1988;40(4):653-670.

101. Matsumoto D, Ekman P. *Japanese and Caucasian facial expressions of emotion (JACFEE) [Slides].* San Francisco, CA: Intercultural and Emotion Research Laboratory, Department of Psychology, San Francisco State University; 1988.
102. Amir N, Taylor CT, Donohue MC. Predictors of response to an attention modification program in generalized social phobia. *J Consult Clin Psychol.* Aug 2011;79(4):533-541.
103. Koster EH, Crombez G, Verschuere B, De Houwer J. Selective attention to threat in the dot probe paradigm: differentiating vigilance and difficulty to disengage. *Behav Res Ther.* Oct 2004;42(10):1183-1192.
104. Oliveira LA, Oliveira L, Joffily M, et al. Autonomic reactions to mutilation pictures: positive affect facilitates safety signal processing. *Psychophysiology.* Jul 2009;46(4):870-873.
105. Hofmann SG. Trait affect moderates cortical activation in response to state affect. *Int J Psychophysiol.* Mar 2007;63(3):258-264.
106. Amodio DM, Harmon-Jones E. Trait emotions and affective modulation of the startle eyeblink: on the unique relationship of trait anger. *Emotion.* Feb 2011;11(1):47-51.
107. Lang PJ, Bradley MM, Cuthbert BN. Emotion, motivation, and anxiety: brain mechanisms and psychophysiology. *Biol Psychiatry.* Dec 15 1998;44(12):1248-1263.
108. Berntson GG, Bigger JT, Jr., Eckberg DL, et al. Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology.* Nov 1997;34(6):623-648.
109. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation.* Mar 1 1996;93(5):1043-1065.
110. Nusslock R, Almeida JR, Forbes EE, et al. Waiting to win: elevated striatal and orbitofrontal cortical activity during reward anticipation in euthymic bipolar disorder adults. *Bipolar Disord.* May 2012;14(3):249-260.
111. Bar-Haim Y, Lamy D, Glickman S. Attentional bias in anxiety: a behavioral and ERP study. *Brain Cogn.* Oct 2005;59(1):11-22.
112. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research.* 1996;29(3):162-173.
113. Boynton GM, Engel SA, Glover GH, Heeger DJ. Linear systems analysis of functional magnetic resonance imaging in human V1. *J Neurosci.* 1996;16(13):4207-4221.
114. Skudlarski P, Constable RT, Gore JC. ROC analysis of statistical methods used in functional MRI: individual subjects. *Neuroimage.* 1999;9(3):311-329.
115. Littell RC, Pendergast J, Natarajan R. Modelling covariance structure in the analysis of repeated measures data. *Stat.Med.* 2000;19(13):1793-1819.
116. Development Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Vienna, Austria; 2012.
117. Neter J. *Applied linear regression models*. Chicago: Irwin; 1996.
118. Westland JC. Lower bounds on sample size in structural equation modeling. *Electron. Commer. Rec. Appl.* 2010;9(6):476-487.
119. MacCallum RC, Widaman KF, Zhang S, Hong S. Sample size in factor analysis. *Psychological Methods.* 3/1999 1999;4(1):84-99.
120. Lenth RV. Java applets for power and sample size. http://www.stat.uiowa.edu/~rlenth/Power. Accessed 10/16/2012, 2012.
121. Bollen KA. Latent variables in psychology and the social sciences. *Annu Rev Psychol.* 2002;53:605-634.
122. Holst K, Bustz-Joergensen E. Linear Latent Variable Models: The lava-package. *Computational Statistics.* 2012.