AN FMRI STUDY EXAMINING THE EFFECTS OF ACUTE D-CYCLOSERINE ADMINISTRATION ON BRAIN ACTIVATIONS AND COGNITIVE FUNCTIONING IN SPIDER PHOBIA

BY

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Abstract

Specific phobias are among the most commonly diagnosed psychiatric disorders. Exposure and Response Prevention (ERP) has become the treatment of choice for specific phobias, and is believed to operate on the basis of fear extinction. Animal studies have shown that acute administration of D-cycloserine (DCS) prior to exposure to a feared stimulus enhances extinction of that fear. Clinical studies in humans have recently demonstrated that DCS facilitates the effects of ERP therapy, presumably through enhancement of memory encoding and consolidation. However, the neural mechanisms underlying these potential benefits of DCS are not understood. The current study used fMRI to examine brain function in subjects with specific phobia and healthy control participants, with and without DCS. The primary objectives of this study were to examine the effects of DCS on 1) neural activity during phobic symptom provocation and 2) neuropsychological functioning.

Results provide evidence that DCS enhances activity in prefrontal cortex (PFC), anterior cingulate cortex (ACC), and insular cortex during phobic symptom provocation. This suggests that DCS may enhance cognitive control and interoceptive integration during emotional processing. Neuropsychological assessment provided evidence that specific phobia is associated with subtle differences in cognitive functioning, most notably on decision-making and strategic organization. DCS also had an effect on cognitive functioning, but the direction of influence depended upon clinical anxiety symptoms.

The current study is the first investigation of acute DCS effects on neural processing during phobic symptom provocation. It is also the first study to examine acute DCS effects on neuropsychological functioning. Results provide direction for future research examining the use of acute DCS administration in enhancing fear extinction, exposure therapy, and cognitive functioning in general.

Chapter 1: Introduction and Background

Specific phobias – anxiety disorders involving persistent fear and avoidance of specified objects or situations (e.g., spiders) – are among the most commonly diagnosed psychiatric disorders, with lifetime prevalence rates of 11.3% in the United States. The cognitive behavioral therapy, Exposure and Response Prevention (ERP), has become the treatment of choice for specific phobias. ERP involves systematic and repeated exposure to a feared or anxiety-provoking stimulus, leading to habituation and extinction of the fear response. Animal models of fear extinction have shown that acute administration of D-cycloserine (DCS) prior to exposure to a feared stimulus enhances extinction of that fear. Clinical studies in human subjects with various anxiety disorders (phobia, social anxiety disorder, panic disorder, and obsessive-compulsive disorder) have also demonstrated that DCS facilitates the effects of ERP therapy. Research with animals, as well as human clinical populations, has also demonstrated that DCS can enhance aspects of cognitive functioning, including memory. Because of this research, current theories postulate that DCS facilitates fear extinction by enhancing the learning process and increasing consolidation of memories. However, the neural mechanisms underlying this process are not understood. Functional magnetic resonance imaging (fMRI) provides a means to measure functional changes in the brain during symptom provocation and memory processing. The current study used fMRI to examine patients with specific phobia and healthy control subjects, with and without DCS. The primary objectives of this study were to provide important information regarding neural mechanisms underlying symptom expression in specific phobia, acute DCS effects in general, and DCS treatment facilitation in particular. The current study also conducted cognitive assessment with these same populations, with and

without DCS, to further elucidate the effects of DCS on human functioning and the mechanisms through which it may enhance ERP therapy. This study therefore aimed to accomplish the following Specific Aims:

1) Measure brain activation during exposure to phobic-related stimuli in untreated spider phobics and healthy control subjects. This aim was critical for defining general group differences in brain function during a clinically specific process (symptom provocation).

Group differences were assessed regarding fMRI signal change during exposure to phobic-related pictures (spiders) vs. neutral pictures (butterflies) in spider phobic patients and healthy control subjects that had not received DCS. It was hypothesized that the placebo spider phobic group would show greater activation than the placebo healthy control group in the following brain regions: insula, hippocampus, orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (dlPFC), anterior cingulate cortex (ACC), and amygdala.

2) Measure brain activation during exposure to phobic-related stimuli in spider phobics and healthy controls, following acute administration of DCS or placebo.

Group differences were assessed regarding fMRI signal change during exposure to phobic-related pictures (spiders) vs. neutral pictures (butterflies) in spider phobic patients and healthy control subjects who had been administered DCS or placebo. Based on previous research and theoretical models related to fear extinction and phobia, it was expected that the phobic DCS group would show increased activations in the medial PFC (mPFC; including the ACC and the OFC), dlPFC, and the hippocampus but decreased activations in the lateral and medial amygdala and insula compared to the phobic placebo group and both healthy control groups.

3) Measure cognitive functioning in spider phobic patients and healthy controls, following acute administration of DCS or placebo.

Group differences were assessed regarding aspects of cognitive functioning shown in previous research to be affected by DCS administration. The aspects of cognitive functioning assessed included: verbal memory, nonverbal memory, executive functioning, and decision-making. Based on previous research, it was expected that subjects administered DCS would perform better than those administered placebo.

To accomplish these specific aims, the proposed study recruited individuals with and without spider phobia from campuses at the University of Kansas (KU), the University of Kansas Medical Center (KUMC), the Kansas City Center for Anxiety Treatment (KCCAT), and the general community. Initial assessment obtained information regarding exclusion/inclusion criteria, psychiatric diagnoses, and severity of spider phobia. Subjects meeting study criteria were scheduled to undergo medication administration, fMRI, and neuropsychological assessment at one time point. Subjects were pseudo-randomized to receive one 100 mg dose of D-cycloserine or matching placebo. Within 2-3 hours after medication administration, subjects underwent fMRI scanning, in which a symptom provocation paradigm was completed. After scanning, a battery of neuropsychological tests was completed with the subjects to assess cognitive functioning.

The proposed study examined the effects of DCS administration on only one aspect of the fear extinction process – the initial exposure to phobic stimuli. However, this study represents the first step in identifying the neural mechanisms through which DCS influences fear extinction and ERP therapy. This work is exploratory and at an early stage of development, but

results are potentially of great importance to the future understanding and treatment of anxiety disorders.

Specific Phobia

Specific phobias are among the most commonly diagnosed psychiatric disorders, with estimated lifetime prevalence rates of 10-15% in the United States (Magee et al., 1996). Specific phobias are defined as "persistent fears that are excessive or unreasonable, cued by the presence or anticipation of specific objects or situations" (American Psychiatric Association, 2000). Spider phobia, or arachnophobia, is a subtype of specific phobia in which the feared "objects" are spiders. It has been estimated that the prevalence of spider phobia in the U.S. population is 5.6% for females and 1.2% for males (Fredrikson, Annes, Fischer, & Wik., 1996). Three primary aetiologies of spider phobia have been proposed: 1) direct classical conditioning, 2) vicarious learning through observation of another individual's conditioning experience, and 3) through transmission of information or instruction related to a stimulus. Dramatic variations are found in the percentage of people attributing their spider phobia to each of these three categories and these variations may depend upon what questionnaire is used (the Origin's Questionnaire [OQ] versus the Phobic Origins Questionnaire [POQ]). Anywhere from 6% to 48% of animal phobias have been attributed to conditioning experiences (negative experience related to the phobic stimulus) while 6% to 55% are due to vicarious experiences, and 3% to 60% developed from transmission of information (Kirby, Menzies, Daniels, & Smith, 1995; Ost, 1987). Differences in findings related to the two questionnaires may be due to underestimation by the OQ and overestimation by the POQ regarding occurrence of conditioning events in the aetiology of phobia (Kirby et al., 1995). Although these findings do

not provide much direction regarding the true percentage of cases that have developed from each type of aetiology, they do make the point that phobia can result from a variety of origins.

The cognitive behavioral therapy, Exposure and Response Prevention (ERP), has become the treatment of choice for anxiety disorders, including specific phobia (Barlow, 2002; Davey, 1997). ERP involves systematic and repeated exposure to the anxiety-provoking stimulus and focuses on decreasing avoidance of situations or objects related to that stimulus. Clinically significant improvement is estimated to occur in 74-95% of patients who complete one- to five-session exposure therapy (Chambless & Woody, 1990; Hellstrom & Ost, 1995; Ost, 1997). Research indicates that one session treatment may be as successful as more extended treatment (Ost, 1996). However, ERP produces stress and discomfort and requires high motivation to undergo treatment. This is most likely the reason that as many as 24% of patients have been reported to drop out of treatment in clinical outcome trials (Marks, Kenwright, McDonough, Whittaker, and Mataix-Cols, 2005). Additionally, phobias will sometimes reappear at a later time or if the patient encounters the stimulus in different contexts (demonstrating lack of generalization). Exposure and Response Prevention is also used in the treatment of other anxiety disorders, including obsessive-compulsive disorder, panic disorder, post-traumatic stress disorder, and others. ERP therapy with these disorders requires more time and often proves difficult for patients to complete (Foa, 1996). Advances made in ERP for phobia would have implications for the treatment of other anxiety disorders as well. Therefore, further advances are needed to increase the tolerance, efficiency, and maintenance of this therapy. DCS has shown some initial promise in this regard.

Functional Neuroimaging Methods

The specific aims and hypotheses of the current study are based upon results from previous research examining neurophysiological correlates of phobia and fear. Cognitive and emotional activation paradigms are used during functional brain imaging to investigate these correlates in human subjects. During activation paradigms, subjects are asked to complete a task that is thought to invoke a particular mental state (emotional or cognitive). The activations associated with this mental state can then be examined within subjects by comparing it with a similar task that is not thought to invoke that particular mental state. However, brain activations associated with the same task can also be compared between two groups of people that differ on a particular characteristic that is thought to affect the mental state of interest. A summary of the functional imaging methods most often used for cognitive and emotional activation studies will be provided to serve as a foundation for understanding the literature. Although there are other functional imaging methods available today, the current discussion will focus on positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), which are most frequently used in the study of phobia and fear.

Positron Emission Tomography. For PET, a biochemical is labeled with a radioactive substance and administered to the subject. When the radioactive isotopes decay within the body, positrons are emitted. The positrons subsequently collide with an electron, producing two gamma rays, or photons, moving in opposite directions (Heiss et al., 1985; Kolb & Wishaw, 1996). The PET scanning device detects the pairs of photons and determines the location of their source in the brain. Detecting the amount of gamma rays in various brain regions provides a measure of the labeled biochemical, which in turn allows for an indirect

measure of regional cerebral blood flow (rCBF; Heiss et al.). Oxygen-15 (^{15}O) is the most frequently used radioactive tracer for the detection of rCBF. The vast majority of PET studies to be discussed utilized ^{15}O as the radioactive isotope, though it should be noted that carbon-11 (^{11}C) and flouro-2-deoxy-D-glucose (^{18}FDG) are sometimes used in other research protocols.

^{15}O has a short half-life of 123 seconds and allows for multiple scans to be acquired during a testing session (approximately 12-15 scans). This is critical for cognitive activation studies in which blood flow is compared between two or more mental states. However, sufficient time must be provided between scans to allow for tracer washout and decay (approximately 10-12 minutes). The duration of data acquisition within a scan is approximately 100 seconds, measured in time frames varying from 10 to 30 seconds in duration (Holmes, 1995).

Magnetic Resonance Imaging. Magnetic Resonance Imaging (MRI) has a powerful magnetic field that causes protons of hydrogen nuclei to align their spins parallel with the magnetic field (Huettel, Song, & McCarthy, 2004; Kolb & Wishaw, 1996). Radiowaves are generated from an electrical coil within the MRI scanner that excites these protons, causing them to flip their spins away from the direction of the magnetic field. When the protons relax to align parallel with the magnetic field, they emit electromagnetic waves. MRI scanner electric coils are able to detect these electromagnetic waves. Proton density and proton relaxation times determine the intensity of the MRI signals (Huettel et al., 2004). Proton relaxation from the excited state has two components: one along the z, or longitudinal axis (T1) and one alone the x-y, or transverse axis (T2). With MRI, different radio wave pulse sequences can be used to emphasize either T1 or T2 contrast.

There are several different MRI techniques used to measure brain function, including dynamic contrast, blood oxygen level-dependent (BOLD), diffusion, and magnetic resonance spectroscopy. This discussion will focus on BOLD fMRI because it is the technique used in the majority of cognitive and emotional activation studies, including all of the fMRI studies to be discussed in relation to specific phobia. fMRI takes advantage of the differential magnetic properties of oxygenated and deoxygenated hemoglobin to indirectly measure brain function (Huettel et al.; Kolb & Wishaw, 1996). Deoxygenated hemoglobin has a much greater magnetic susceptibility, or intensity of magnetization when in a magnetic field, than oxygenated hemoglobin. Changes in blood flow and oxygenation level of the blood lead to an increase in the T2 MR signal in response to neuronal activity. Therefore, measuring T2 MR signal changes in response to cognitive tasks allows for an indirect measure of the associated neuronal activity by quantifying hemodynamic properties of brain regions.

During an fMRI scanning session, several runs of functional images are acquired, each lasting 5-10 minutes. Within each run, data are acquired as a time series of *volumes*, which are images created from a number of image *slices* taken throughout the brain (Huettel et al.2004). These slices are composed of data acquired from individual voxels. In a given fMRI experiment, there may be any number of runs, each consisting of approximately 100-400 volumes. Each volume is composed of multiple slices, which in turn consists of a 64X64 or 128X128 matrix of voxels. Voxel size is usually cubic (e.g. 3.75mm long X 3.75 mm wide X 3.75 mm thick) though non-cubic can also be utilized (Huettel et al.).

There are two basic types of fMRI designs: blocked designs and event-related designs (Huettel et al., 2004). In a blocked design, 2 or more alternating conditions and, in

most cases, a baseline condition, are presented. Each stimulus block is approximately 10 to 30 seconds in duration. In event-related designs, stimuli are presented as individual events. Changes in fMRI signal after the occurrence of an experimental event are measured. Blocked design is the method used most frequently when investigating cognitive processes that cause prolonged changes in emotional or cognitive states. Event-related fMRI is used to measure more discrete, short-term cognitive processes.

Both PET and fMRI data analysis can be conducted on a voxel-by-voxel basis or for specified regions of interest (ROIs). Voxelwise analysis involves statistical tests of each separate voxel, whereas ROI analysis involves statistical testing of discrete regions of voxels (Huettel et al., 2004). Although power may be decreased when conducting voxel-by-voxel analysis, it allows for inclusion of all brain regions. ROI analysis increases power but requires more time and effort then voxelwise analysis. Additionally, if only ROI analysis is performed, potentially significant activations in other regions will not be detected. The current trend is to include both voxelwise and ROI analyses.

fMRI is often preferred to PET because it does not require radioactive substance to be injected into the subject and has superior temporal and spatial resolution (Huettel et al., 2004). Although fMRI is currently the preferred method for measuring brain activations associated with cognitive or psychological processes, PET and fMRI do have high convergent validity (Volkow, Rosen, & Farde, 1997). Therefore, results reported from PET and fMRI studies can be consolidated to develop theories of brain function. In the following section, PET and fMRI research examining correlates of phobia symptom provocation and fear extinction will be discussed. If not otherwise specified, the PET studies utilized ^{15}O as the radioactive

label and the fMRI studies measured the BOLD response. Variations in imaging methodology will be discussed when relevant to the research findings.

Functional Imaging Studies of Fear and Phobia

Neuroanatomical abbreviations frequently used within this manuscript are listed in Table 1.

Positron Emission Tomography. Functional imaging studies, utilizing PET or fMRI, have been conducted with patients suffering from specific phobia, measuring brain activity during exposure to fear-inducing stimuli. The majority of these studies have involved only spider phobia, though snake phobia, small animal phobia, and blood-injection-injury phobia have also been examined. The first imaging study with specific phobia was published by Mountz et al. in 1989. These researchers utilized PET to examine brain activations of small animal-phobic and non-phobic subjects when exposed to feared animals (snake, spider, or rat depending upon the subject's particular phobia). For symptom provocation, the feared animal was held at eye level in front of the subject. However, during the PET scan, subjects were instructed to close their eyes, though the stimuli remained in the same location. In comparison with a resting state baseline measure (phobic stimuli not in the room), exposure to phobic stimuli did not induce significant differences in brain activations. Additionally, there were no significant differences in activation when comparing phobic and control subjects. The lack of significant findings in this study could have been due to several factors. It could be the phobic stimuli were not provocative enough to elicit a response. However, this is unlikely considering the fact that subjects' reports of anxiety level, as well as heart rate measurements, differed significantly between the phobic and control conditions. Another factor could be that stimulus presentation time was longer in duration (6 minutes) than many of the subsequent PET studies.

Additionally, it is not clear what duration of image acquisition was utilized. If data were acquired over a longer period of time, this could have had an effect on the findings due to washout of the radioactive tracer or habituation to the stimulus. Lastly, only ROI analyses were conducted by Mountz et al. whereas voxelwise analysis was included in many of the subsequent studies. Depending upon the size and placement of the ROIs, this method may have been less sensitive in detecting activation changes. Although the exact reason for insignificant findings in this first study is unknown, a multitude of studies conducted since have reported significant activation in response to phobic provocation.

Utilizing a PET paradigm similar to that of Mountz, Rauch et al. (1995) examined functional activations of small animal-phobic subjects exposed to phobic stimuli. The phobic paradigm consisted of animals within clear cages, which the subjects touched during scanning. Functional activation during the phobic condition was compared to activations during a control condition (exposure to an empty cage) utilizing both voxelwise analysis and ROI analysis. Results indicated that exposure to phobic stimuli was associated with increased activations in the anterior cingulate cortex (ACC), left insula cortex, left somatosensory cortex, left orbitofrontal cortex (OFC), left thalamus, and right anterior temporal cortex. Similar results were reported for a combined group of simple phobia, obsessive-compulsive disorder, and post-traumatic stress disorder patients during provocation paradigms individualized for each patient (Rauch, Savage, Alpert, Fishcman, & Jenike, 1997). In this study, there were increased activations in the right inferior frontal cortex, right posterior medial orbitofrontal cortex, bilateral insula cortex, bilateral lenticulate nuclei, and bilateral brain stem. It should be noted that Fredrikson, Wik, Annes, Ericson, and Stone-Elander (1995) reported *decreased* activations

in many of these same regions, including the hippocampus, posterior cingulate, prefrontal, orbitofrontal, and temperopolar cortices during exposure to phobic versus neutral stimuli. (These activation changes were detected through ROI analysis; voxelwise analysis was not conducted.) These reports regarding decreased limbic and frontal activations contradict those of Rauch et al. (1995; 1997) and subsequent PET and fMRI studies, as will be discussed more in depth at the end of this section.

Carlsson et al. (2004) utilized PET to examine brain activations of spider or snake phobics in response to phobic as well as non-phobic, fear-relevant *picture* stimuli. This study utilized a masking paradigm in which neutral stimuli were displayed directly after each phobic or non-phobic, fear-relevant stimuli. In one condition, a very short period of time was included between the experimental and neutral stimuli (14 ms), whereas for the other condition, a longer period of time was included (308 ms). Differences in activation for the "short" and "long" conditions were compared, assuming that the long condition allowed for full visual processing while the short condition did not. During the short condition, there was left amygdala activation in response to fear-relevant (both phobic and non-phobic) stimuli. However, when exposure time allowed for complete visual processing, the amygdala activated bilaterally in response to phobic-relevant stimuli and showed no activation in response to non-phobic, fear-relevant stimuli. During the long condition, subjects also showed increased activation in the ACC, OFC, anterior insula, and the periaquaductal gray (PAG).

An additional PET study exposed spider phobic subjects to picture stimuli for longer periods of time than previous studies in order to examine habituation effects (Veltman et al., 2004). Voxelwise analysis indicated that, compared to non-phobic subjects, spider phobic

subjects showed increased activations in the left fusiform gyrus and right parahippocampal gyrus when presented with phobic stimuli versus neutral stimuli (butterflies). Also reported were signal decreases as a function of time (unique to phobic stimuli) in the medial temporal lobe, including the posterior insula cortex, hypothalamus, and right amygdala.

Functional Magnetic Resonance Imaging. The majority of studies conducted in recent years to examine neurophysiological responses to phobic stimuli have used fMRI as opposed to PET. Dilger et al. (2003) used fMRI to examine brain activations of spider phobic subjects and non-phobic controls to pictures of spiders, snakes, and mushrooms. Voxelwise analysis showed that, for spider phobic subjects but not controls, there was significant activation in the left amygdala and the right and left insula in response to spider pictures as compared to both snake and mushroom pictures. The researchers also examined between-group differences in brain activations, revealing spider phobic subjects to have stronger activations in the right and left insula, right OFC, and the posterior cingulate cortex in response to spider pictures, but not to snake or mushroom pictures. Schienle, Schafer, Walter, Stark, and Vaitl (2005) examined neural correlates of phobia-relevant, generally fear-inducing, disgust-inducing and affectively neutral pictures in both spider phobic and non-phobic individuals. The patient group showed greater activation to phobic-relevant stimuli in the visual association cortex, amygdala, right dlPFC, and right hippocampus. A neuroimaging study examining the neural correlates of *anticipatory anxiety* in spider phobia (comparing anticipation of phobic stimuli to anticipation of neutral stimuli) implicated the dorsal ACC, insula, thalamus, visual area,

and a region of the extended amygdala—the bed nucleus of the stria terminalis (BNST; Straube et al., 2007).

fMRI has been used not only to examine differential activation to phobic stimuli for phobic and non-phobic subjects, but also to examine the effects of CBT on brain activations. Paquette et al. (2003) used a paradigm that included videos depicting live spiders or live butterflies. Spider phobic subjects had increased activations as compared to control subjects within the dorsolateral prefrontal and the parahippocampal gyrus, as detected through voxelwise analysis. By repeating the same fMRI paradigm with the spider phobic patients after completion of CBT, these researchers demonstrated that therapy resulted in normalization of these activations. However, a phobic control group (receiving no therapy) was not included in this study for comparison.

Straube et al. (2006) used videos of a moving spider and of a moving, black, synthetic cylinder to represent phobic and neutral stimuli (respectively) during fMRI. After the initial scan, spider phobic subjects were randomized to a CBT or a wait-list control group, after which the same fMRI paradigm was repeated. Data analysis included both ROI (dorsolateral prefrontal cortex, inferior frontal gyrus, insula, amygdala, and posterior cingulate cortex) and voxelwise analysis. For the pre-treatment scan, increased activations were found in the insula and ACC for phobic subjects as compared to control subjects in response to phobic stimuli (versus neutral stimuli). These activations were reported to have normalized at the post-treatment scanning session for subjects provided CBT, but not for the wait-listed phobic group. Another more recent event-related fMRI study also reported normalization of activity in the amygdala, as well as the ACC and insula (in response to symptom provocation), after CBT

treatment (Goossens et al., 2007). The most recent fMRI treatment study in spider phobia reported normalization (increased) of activity in the OFC from pre- to post- CBT treatment (Schienle et al., 2007). In this study, decreases in the amygdala and insula were positively correlated with decreases in phobia symptoms.

fMRI and Fear Extinction in Healthy, Non-Phobic Subjects. In recent years, there has been an upsurge in human neuroimaging studies involving fear conditioning and extinction paradigms. This is most likely due to the progress made in animal studies of fear conditioning and extinction (discussed below), and the focus in psychology on translational research. In classical fear conditioning paradigms, subjects (animal or human) are made to fear a previously neutral stimulus (conditioned stimulus [CS]) through concurrent presentation with a noxious stimulus (unconditioned stimulus [UCS]). Fear extinction involves repeated exposure to the CS without concurrent presentation of the UCS. The fear extinction process is conceptualized as involving not an "unlearning" of the previous association, but a learning of a new, more neutral association. This new association therefore competes with the old CS-UCS association, making it appear weaker and reducing the physiological and behavioral response to the CS (Ledgerwood, Richardson, & Cranney, 2003). Most of what we know regarding fear conditioning and extinction come from animal research, which will be discussed more in depth in a later section. However, the following studies provide some information regarding the neural mechanisms underlying fear extinction in humans.

The fear conditioning paradigm utilized by Phelps, Delgado, Nearing, and Ledoux (2004) consisted of pairing colored squares with a mild shock to the wrist. ROI analysis was used to examine activations within various regions of the amygdala and medial prefrontal cortex

(mPFC). Three regions of the mPFC showed differential activations in response to the conditioned stimulus as opposed to a neutral stimulus during fear acquisition, as well as on the first (Day 1) and second day (Day 2) of extinction training. During conditioning, the dorsal region of the bilateral anterior cingulate showed increased activation in response to the conditioned stimulus whereas the more ventral region of the ACC and the medial frontal gyrus of the mPFC showed decreased activations during presentation of the CS as compared to a neutral stimulus. The dorsal anterior cingulate showed increased activation during all three phases, though the level of activation was not correlated with the conditioned response. In the amygdala, there was increased activation to the CS during acquisition. However, the amygdala response switched during the first day of extinction, showing less activation in response to the CS as compared to the neutral stimulus, and then normalized during Day 2 of extinction training (Phelps et al., 2004). Another fMRI study examining brain activations associated with fear conditioning and fear extinction (Gottfried and Dolan, 2004) reported that, during conditioning, the rostromedial OFC, dorsomedial amygdala, and insula cortex showed significant activations in response to a CS as compared to a neutral stimulus. At extinction, the caudal OFC, right lateral amygdala, and the ventromedial PFC showed increased activations in response to the CS. However, activations in the medial amygdala and the rostromedial OFC were decreased in the extinction trial as compared to the conditioning trial (Gottfried & Dolan, 2004). Dunsmoor, Bandettini, and Knight (2007) varied the rate at which a CS was paired with an UCS (from intermittent to constant) and found that activations within the ACC and amygdala increased as the pairing rate increased. In contrast, the dlPFC and insula were

activated only during intermittent pairing rates. These results would suggest that the dlPFC and insula are involved in the processing of uncertainty regarding fear or conditioning.

Most recently, Milad et al. (2007) specifically examined the involvement of the dorsal ACC in fear conditioning. The authors reported the dorsal ACC to have greater activation during presentation of a stimulus that had been paired with an unconditioned stimulus (CS+) than one that had not (CS-). Additionally, dorsal ACC activation correlated with skin conductance response during CS+ presentation. Two studies (one by the same group who authored the last study discussed—Milad et al., 2007 and Kalisch et al., 2006) investigated the neural correlates of fear extinction *recall*. Both studies reported involvement of the ventromedial PFC (vmPFC) and hippocampus in extinction recall, which were correlated with one another, and seemed to be particularly important for recall of contextual information regarding extinction (Milad et al., 2007).

Researchers have recognized that, although fear conditioning provides a good approximate model of phobia, it is not perfect. As discussed before, the majority of people with phobia do not report having *personally experienced* aversive conditioning to the feared object or animal. There may be evolutionary and genetic influences at work, in which humans with a fear of certain animals/objects were able to survive (along with their genetic material). This, in turn, caused descendants to be "programmed" with this same fear (Rakison & Derrnger, 2008). This would suggest that those of us without phobia simply extinguished the programmed fear whereas those with phobia did not experience extinction. Additionally, humans have an ability to learn through *verbal exchange* of information as well *vicariously* through others' experiences (as discussed above). Creative neuroimaging studies have been conducted to

address this issue and examine distinct neural mechanisms of fear that is learned through direct experience, observation, or verbal information. In one such study (Phelps et al., 2001), it was verbally communicated to subjects that, during fMRI scanning, presentation of a certain colored block would be associated with a shock whereas presentation of another color would not involve shocks. However, no shock was ever provided. Regions of the amygdala and insula activated in response to the expected CS+ more than during the other conditions and amygdala activation correlated with skin conductance response. Additionally, amygdala response attenuated (or habituated) over time so that, by the end of the paradigm, amygdala activation for the two types of stimuli were equal. Although this paradigm could also be viewed as an anticipatory anxiety paradigm, it is interesting to find that expectation of aversive stimuli—communicated through verbal information only—is associated with modulation of activity in the amygdala and insula. This same lab conducted another study (Olsson, Nearing, and Phelps, 2007) in which subjects were shown videos of other individuals undergoing fear conditioning to certain stimuli (as a model of vicarious learning). After viewing these videos, fMRI scanning was conducted in which the same stimuli were presented. Increased amygdala activation was found for the stimuli used as CS+ in the video. Therefore, it seems that the amygdala is involved in the experience of fear regardless of the type of fear learning that occurred.

Summary of Imaging Studies with Phobia and Fear Extinction. The report of decreased activations in frontal and limbic structures reported by Fredrikson et al. (1995) is contradictory to the results of other PET (Rauch et al. 1995; 1997) and fMRI (Dilger et al., 2003; Paquette et al., 2003; Straube et al., 2006) studies showing primarily increased activations in these regions.

The inconsistencies can partly be explained by the fact that activations within the frontal cortex, as measured by PET, have been reported to vary depending upon the behavioral reaction of the subject (Johanson et al., 1998). In the study by Johanson et al., the ^{133}Xe inhalation method was used. This method allows information to be collected regarding superficial cortical areas only, excluding analysis of deeper brain structures. Results showed that subjects displaying panic reactions had decreased activations in the frontal cortex whereas those who did not display panic reactions had increased activations. It has been hypothesized that decreased activations in frontal and limbic structures may represent reduced conscious cognitive processing associated with a defensive response to feared stimuli whereas increased activations in limbic and frontal networks may be associated with the use of cognitive strategies for coping with exposure to feared stimuli (Paquette et al.). It has additionally been suggested that habituation effects during scanning could be responsible for the inconsistent results, as Veltman et al. (2004) demonstrated that amygdala, insula, and hypothalamus activations in response to phobic stimuli habituated significantly after approximately 15-25 minutes of exposure. Although habituation may not be the only reason for inconsistent results, it is interesting to note that Fredrikson et al. (1995) used film clips that were four minutes in duration to serve as the phobic stimulus. The other PET and fMRI studies using provocation paradigms presented stimuli for approximately one minute and this time was often broken up into blocks of stimuli presentation lasting only 10-25 seconds each. By including a period of rest or neutral stimulation between phobic stimuli presentations, these studies could have decreased the effects of habituation.

The results of PET and fMRI studies in specific phobia have most consistently identified activations (signal increases during symptom provocation) in the amygdala, insula, medial prefrontal cortex (including the ACC and the OFC), and dorsolateral prefrontal cortex (dlPFC; Carlsson et al., 2004; Dilger et al. 2003; Fredrikson et al., 1995; Johanson et al., 1998; Rauch et al., 1995; Straube et al., 2004; Wik et al., 1996). Other areas that have been reported to activate in response to phobic stimuli include the temporal cortex, fusiform gyrus, hippocampus, parahippocampal gyrus, thalamus, putamen, periaqueductal gray, and the posterior cingulate (Carlsson et al., 2004; Dilger et al. 2003; Fredrikson et al., 1995; Johanson et al., 1998; Paquette et al., 2003; Rauch et al., 1995; Straube et al., 2004; Veltman et al., 2004; Wik, Fredrikson, & Fischer, 1997). A recent meta-analysis of neuroimaging studies in phobia, social anxiety disorder and PTSD reported the most consistent findings to be increased insula and amygdala activation (Etkin & Wager, 2007). Evidence has been provided that successful cognitive behavioral therapy normalizes activations in response to phobic stimuli in the ACC and insula (Straube et al., 2006; Goossens et al., 2007), and also in the DLPFC, parahippocampal gyrus (Paquette et al.) and OFC (Schienle et al., 2007). Fear conditioning in non-clinical human populations have revealed increased activations in the ACC, amygdala, dlPFC, insula, and OFC (Phelps et al., 2004; Dunsmoor et al., 2007; Gottfried & Dolan, 2004; Milad et al., 2007). Fear extinction or "habituation" processes may be associated with decreased activations within the amygdala, OFC, and possibly insula (Gottfried & Dolan, 2004; Phelps et al., 2004; Veltman et al.) while the vmFC and hippocampus have been demonstrated to play a role in memory for fear extinction (Kalisch et al., 2006; Milad et al., 2007).

Animal Studies Examining Neural Mechanisms of Fear Conditioning and Extinction

Animal models have contributed significantly to our understanding of anxiety disorders, especially regarding cued anxiety responses. There is a large animal literature utilizing classical fear conditioning as a model for phobia and fear extinction as a model of exposure therapy (McAllister & McAllister, 1971; Uys, Stein, Daniels, & Harvey, 2003). There have been many excellent reviews conducted recently regarding this literature and translation into the clinical treatment of anxiety disorders (Hofmann, 2007a, 2007b; McNally, 2007; Quirk & Mueller, 2008). Similar to findings reported in the neuroimaging studies described above, animal research has indicated that the amygdala, along with the hippocampus and prefrontal cortex, are important in the process of emotional learning and fear extinction (Myers & Davis, 2002). Single-cell recording studies, as well as studies examining effects of induced brain lesions have provided support for these conclusions.

Animal research has found that the amygdala, particularly the basolateral complex of the amygdala (BLA), is important in emotion and the experience of fear. Lesioning of the amygdala leads to profound effects on emotional behavior, reducing vigilance and weariness of previously feared objects (Davis & Whalen, 2001; Kluver & Bucy, 1939; Weiskrantz, 1956). Fear conditioning seems to depend, at least partially, on NMDA receptor activation within the BLA (Baldwin, Holahan, Sadeghian, & Kelley, 2000; Davis & Whalen, 2001; Miserendino, Sananes, Melia, & Davis, 1990) and the BLA seems to be particularly important for the maintenance of fear once it has been acquired (Anglada-Figueroa & Quirk, 2005). Additionally, stimulation of the BLA can produce behavioral and autonomic changes that resemble a state of fear (Davis & Whalen, 2001). Although research on anxiety and fear in

animal studies has primarily focused on the amgydala and its connections, a related region, the bed nucleus of the stria terminalis (BNST) is considered part of the "extended amygdala" region and may be involved in the more long-term experience of fear, which could more closely resemble the human "anxiety" experience (Davis et al., 1997; Davis & Whalen, 2001; Rosen & Donley, 2006).

The hippocampus has also been implicated as an important structure for fear, especially regarding the extinction process. Fear conditioning and extinction can still occur when the hippocampus has been lesioned, but it cannot be maintained (Maren, Aharonov, & Fanselow, 1997). Additionally, Corcoran, Desmond, Frey, and Maren (2005) demonstrated that hippocampal inactivation attenuated fear extinction (though not completely blocking it) and seemed to additionally diminish memory regarding contextual attributes of fear extinction. Therefore, the hippocampus is thought to be important for the memory of fear conditioning as well as fear extinction.

When lesions are made in the prelimbic or infralimbic regions of the medial prefrontal cortex, fear extinction (but not fear conditioning) is hindered in animals (Morgan, Schulkin, & LeDoux, 2003). Additionally, s*timulation* of the mPFC has also been shown to increase retention of extinction whereas mPFC *depression* caused mice to resist extinction (Herry & Garcia, 2002). Single cell recording studies have demonstrated that rats receiving stimulation of the infralimbic cortex subregion of the mPFC (which corresponds to the subcallosal anterior cingulate in humans) during nonreinforced CS exposure respond faster to extinction training and show increased retention of fear extinction (Jinks & McGregor, 1997; Milad, Vidal-Gonzalez, & Quirk, 2004). The dorsomedial and dorsolateral prefrontal cortices have also been

shown to activate along with the ventral medial prefrontal cortex at post-tests of extinction recall (Barrett, Shumake, Jones, & Gonzalez-Lima, 2003). It is believed that these regions of the PFC could modulate fear extinction through the medial prefrontal cortex, or through direct connections with the amygdala (Milad, Rauch, Pitman, & Quirk, 2006). Single-cell recording studies have shown that stimulation of the mPFC causes inhibition of the amygdala's lateral and central nuclei neurons (Quirk, Likhtik, Pelletier, & Pare, 2003). Additionally, single-cell recording studies have demonstrated that neuronal firing of neurons in the lateral nucleus of the amygdala (LA) are reduced during presentation of a previously CS in the absence of the UCS (Maren & Quirk, 2004; Repa et al., 2001).

Animal Studies of D-cycloserine and Fear Conditioning and Extinction

N-methyl-D-aspartate (NMDA) receptors are implicated in the fear conditioning and extinction process based on observations that NMDA antagonists (e.g. D,L-2-amino-5-phosphonovaleric acid [AP5]), injected into the BLA, block the acquisition of fear in fear conditioning paradigms (Davis, 1992; Falls, Miserendino, & Miserendino et al., 1990; Richardson, Ledgerwood, & Cranney, 2004). This effect has been demonstrated using visual, auditory, and olfactory cues in the conditioned fear paradigm (Campeau, Miserendino, & Davis, 1992; Miserendino et al., 1990; Paschall, Walker, & Davis, 2001). However, studies also indicate that intra-amygdala infusions with NMDA antagonists do not disrupt fear-potentiated startle (Campeau et al., 1992; Miserendino et al., 1990; Rodrigues, Schafe, & LeDoux, 2001). This would suggest that NMDA antagonists do not block fear acquisition by inactivating the amygdala nor do they prevent cognitive access and processing of the fear stimulus (Davis, 2002). Administration of NMDA antagonists in the BLA has been shown to

block fear extinction as well as fear acquisition (Falls et al., 1992). However, it does not appear as if the NMDA antagonists damage aspects of the amygdala associated with fear conditioning and extinction. This is demonstrated by the fact that rats previously included in such studies subsequently showed normal fear conditioning and extinction when not administered NMDA antagonists.

Results from the studies described above demonstrate the role of NMDA receptors in fear acquisition and extinction. These findings led researchers to hypothesize that NMDA *agonists* may have the opposite effect –*enhancing* of the fear extinction process. Full NMDA agonists have been associated with neurotoxicity due to deregulation of Ca^{2+}. However, partial NMDA agonists, such as D-cycloserine (DCS), are not known to produce toxicity and have few side effects (Davis, 2002). Walker, Ressler, Lu, and Davis (2002) demonstrated that systematic administration of DCS, as well as direct infusion of DCS into the basolateral nucleus of the amygdala, facilitated the extinction of conditioned fear in rats. In this study, a fear-potentiated startle paradigm was used to examine the effects of 3.25, 15, and 30 mg/kg doses of DCS as compared to saline injections. The fear extinction response was dose-dependent. All DCS groups experienced enhanced fear extinction compared to the saline group and the 15 and 30 mg/kg DCS groups showed greater fear extinction than the 3.25 mg/kg DCS group. The fear extinction enhancement properties of DCS have been replicated by other researchers using a variety of fear-conditioning paradigms (Ho et al., 2005; Ledgerwood, Richardson, & Cranney, 2003, 2004; Yang & Lu, 2005). In these studies, DCS had an effect on fear extinction only when it was paired with extinction training, not when it was administered alone. Ledgerwood, et al. (2003) also demonstrated that DCS can enhance extinction if administered *after*

extinction training, though the effects decreased as the time between extinction training and DCS administration increased. These results were interpreted to indicate that DSC produces effects through enhancing memory consolidation regarding the fear extinction process.

Another concept of fear conditioning in animal paradigms is reinstatement. Animals that undergo fear extinction will subsequently have their fears "reinstated" in response to UCS exposure. Ledgerwood et al. (2004) demonstrated that rats that had undergone fear extinction supplemented with DCS (as compared to saline) did not experience reinstatement. This would indicate that not only does DCS administration enhance the effects of fear extinction, it helps to maintain extinction. Additionally, if two separate conditioned stimuli have been paired with an UCS, rats that undergo DCS-supplemented fear extinction with only one CS will also show extinction of fear associated with the other CS (Ledgerwood, Richardson, & Cranney, 2005). This generalization across the conditioned stimuli does not occur in rats provided only saline prior to fear extinction. Furthermore, rats that have undergone DCS-supplemented fear extinction are not impaired in their ability to acquire fear in the future, even when that fear involves the previous CS or UCS (Ledgerwood et al., 2005). Therefore, not only does DCS enhance fear extinction, it appears to enhance the maintenance and generalization of fear extinction as well. Additionally, it appears that these effects are produced without causing permanent disability regarding fear acquisition and learning.

Human Studies of D-cycloserine and Exposure and Response Prevention

The first study to examine the effects of DCS on ERP therapy did so with height phobia (Ressler et al., 2004). Subjects were randomly assigned to one of three treatment groups: placebo with virtual reality exposure (VRE) therapy (n=10), 50 mg DCS with VRE therapy

(n=8), and 500 mg DCS with VRE therapy (n=9). Each subject underwent two 35-45 minute VRE sessions within a 1-2 week period. Subjects took medication 2-4 hours prior to the therapy sessions. One week following the second VRE session, the groups that received DCS showed significantly less fear of heights, as measured by the subjective units of distress scale (SUDS) at successive elevator floors (as experienced through virtual reality). This difference was observed for both the 50 mg and the 500 mg DCS groups. Twenty-one of the subjects returned for follow-up assessment 3 months later. Again, those treated with DCS-supplemented therapy experienced significantly less fear of heights (as measured by SUDS) than those treated with placebo-supplemented therapy. A similar study was conducted with social anxiety disorder (Hofmann, Pollack, and Otto, 2006a), using 50 mg DCS, administered one hour prior to each of 4 sessions of ERP therapy. The DCS group had greater decreases in social anxiety symptoms (as measured by the Social Phobia and Anxiety Inventory) at post-treatment and one month follow-up. The effect size for the difference at follow-up (Cohen d=1.43) was even greater than that at post-treatment (Cohen d=.98).

Two studies have been published reporting null results for the effects of DCS (Guastella et al., 2007a and 2007b). Both of these studies used non-clinical samples, however. The first (Guastella et al., 2007a) recruited college undergraduates who had a fear of spiders (but not diagnosable as spider phobia) and provided one session of ERP, augmented with either 50mg DCS, 500mg DCS, or placebo. The second study (Guastella et al., 2007b) conducted a differential shock conditioning paradigm with non-anxious control subjects and administered either 50 mg DCS, 500 mg DCS, or placebo prior to fear extinction trials. For both of these studies, there were no significant findings regarding skin conductance or self-report measures

of anxiety. In response to these null findings, many researchers postulated that DCS effects would only be detectable if the population was diagnosable with a clinical anxiety disorder. Otherwise, even just one session of ERP could produce a floor effect across groups in which effects would not be detected. In response to these criticisms, the same lab that reported null findings conducted a study examining the effects of DCS in social anxiety disorder (Guastellla et al., 2008). With four sessions of ERP, those receiving 50 mg of DCS augmentation (1 hour prior to session) had a greater reduction in anxiety symptoms than those provided placebo. This effect was apparent at post- treatment and follow-up, though the effect seizes were lower than that reported by Hofmann et al. (2006a). For the DCS group only, anxiety reduction was related to subjects' appraisals about their speech performances. The authors postulated that adaptive learning and self-appraisal may be potential mechanisms for the effects of DCS.

Three studies have been published thus far examining the effect of DCS-augmented ERP for OCD. The first of these (Storch et al., 2007) conducted 10 weekly sessions of ERP, augmented with 250 mg of DCS, administered 4 hours prior to each session. For a variety of outcome measures (including the Y-BOCS) assessed at pre-treatment, post-treatment, and 2-months follow-up, there were no differences between treatment groups (though both groups benefited significantly from treatment). Kushner et al., (2007) examined the effects of 125 mg DCS versus placebo, administered two hours prior to 10 ERP sessions (conducted twice weekly). Although comparing groups on Y-BOCS scores at mid- and post- treatment revealed no differences, the DCS group did have a faster response to treatment as measured through estimated SUDS ratings of hierarchy items. This effect was only significant at Session 4, however. The groups did not differ at the end of treatment. Interestingly, the DCS group had a

significantly lower dropout rate (approximately 7%) than placebo (approximately 35%). The most recent DCS study in OCD administered 100 mg DCS or placebo one hour prior to each of 10 sessions ERP, conducted twice weekly (Wilhelm et al., 2008). The DCS group had greater decreases in symptoms (as assessed by the Y-BOCS) at mid-treatment and post-treatment, though the effect only reached significance at mid-treatment. In addition, depression scores (as assessed by the BDI) decreased significantly more in the DCS group as measured at post-treatment. Although it was recognized that the effect seen for depressive symptoms may have been secondary to accelerated progress in regards to the OCD symptoms, the authors also suggested that alteration of dysfunctional beliefs may provide a common mechanisms for the enhancing effects of DCS for both depressive and OCD symptoms.

In summary, results from clinical studies with DCS-augmented ERP have been promising. There is some indication that it may not only influence behavior, but also cognitions and appraisals. Therefore, the mechanisms of DCS in clinical treatment may be more complex than what can be measured in animal studies. It will be important for future research to identify the specific cognitive, behavioral, and neural mechanisms for DCS in human populations in order to supplement the knowledge gained from animal studies.

Functional Imaging Studies of D-Cycloserine

To the author's knowledge, there has been only one neuroimaging study investigating the effects of *acute* D-cycloserine administration. Britton et al. (2007) administered 500 mg DCS or placebo to healthy subjects (with no clinical diagnosis) 1.5 hours prior to fMRI scanning. During scanning, subjects viewed either fearful or happy faces during 80-second blocks (surrounded by 20-second fixation blocks). This paradigm has previously been shown to be

associated with initial amygdala activation and subsequent habituation throughout the paradigm. The paradigm was chosen in order to examine the effects of DCS on amygdala activation and habituation. Therefore, fMRI analysis was restricted to the bilateral amygdala. There were no differences found between groups regarding subjective anxiety state. Amygdala activation was decreased in the early period of the paradigm for the DCS group and therefore also showed less habituation in this region than the placebo group. This finding was opposite from what was predicted by the investigators. The authors interpreted this as evidence that DCS may alter the overall level of amygdala activation instead of influencing the speed of amygdala activation. However, it was noted that the paradigm did not allow for high enough temporal resolution to examine the very early amygdala response and therefore the possibility still remains that DCS is associated with exaggerated initial amygdala response that then habituates very quickly. It was also noted that whole-brain analyses were not performed and therefore conclusions cannot be made regarding whether the amygdala is being affected directly or through other brain regions modulating amygdala activity. Further research is needed to examine these possibilities as well as to examine neural effects of DCS in clinical populations.

Network Models of Fear Processing and Extinction

Despite apparent differences in the experience of anxiety and mechanisms for the alleviation of anxiety between humans and animals, the primary neural networks that have been implicated are relatively similar. Neurophysiological models of emotional and, more specifically fear processing, have been proposed by other researchers and most often implicate the amygdala, prefrontal cortex, insula, and ACC (Phan, Wager, Taylor, and Liberzon, 2004).

Brain imaging studies in humans with specific phobia also indicate that these regions become activated during exposure to phobic stimuli. As mentioned before, extinction is currently thought to involve the learning of a new association that competes with an older association. However, the neurophysiological basis of this process is less understood than for fear processing in general. The brain regions hypothesized to play important roles in the extinction process are generally the same regions implicated in fear processing (Phan et al.). The brain regions implicated in fear processing and fear extinction are part of limbic and paralimbic brain networks, thought to comprise affect regulation systems that assign reward and punishment value to stimuli in the environment (Scheinle et al., 2005). The animal and human research relevant to each region will be summarized below to provide a basis for the hypotheses and results presented in the current study.

Amygdala. The amygdala is thought to lie at the center of the brain's emotional processing system (Lang, Davis, & Ohman, 2000). As discussed previously, it has been shown to be involved in both fear acquisition and fear extinction in animals and is correlated with response to feared stimuli in both phobic and non-phobic human populations (Flynn, Benson, & Ardila, 1999). Purportedly, over 60% of studies that have examined fear-processing in non-phobic, healthy control subjects report amygdala activation (Phan et al., 2004). It is believed that after sensory input is relayed through the thalamus, it diverges into two pathways, a direct and an indirect (Flynn et al.). The direct pathway continues directly to the amygdala, allowing immediate processing of potentially dangerous stimuli (Flynn et al.). The existence of such a pathway is illustrated in studies that have reported amygdala activation in response to short, masked stimuli that are not consciously processed by the subject (e.g. Carlsson et al., 2004).

The more indirect pathway is thought to involve the hippocampus, parahippocampal gyrus, and the sensory, association and prefrontal cortices (Flynn et al.). Through this indirect pathway, the significance of stimuli can be more accurately estimated, by allowing the consideration of previous experience and contextual cues.

Neurons in the lateral nucleus of the amygdala are thought to modulate the activity of the neurons in the central nucleus (Davis, 2006). The lateral nucleus connects to the basal amygdala (including basolateral, basomedial, and accessory basal nuclei), which in turn, connects to the central nucleus (Anglada-Figueroa & Quirk, 2005). The basal amygdala is the primary source for projections between the amygdala and prefrontal cortex. Animal research has provided evidence that, although the basal amygdala may not be necessary for the acquisition of fear, it is important in the maintenance of fear (Anglada-Figueroa & Quirk). Although regions of the amygdala have been shown to activate in response to exposure with feared stimuli, it has been demonstrated that the amygdala activations decrease in humans during fear extinction (Gottfried and Dolan, 2004; Phelps et al., 2004).

As noted earlier, DCS has been shown to influence fear extinction in animals when injected directly into the basolateral amygdala (i.e. Walker et al., 2002). Additionally, a neuroimaging study provided evidence that 500 mg DCS decreases activity in the amygdala for non-anxious control subjects during the processing of emotional faces (Britton et al., 2007).

Prefrontal Cortex. It is thought that sensory information enters conscious awareness only after being processed by the prefrontal cortex (Flynn et al., 1999). The prefrontal cortex is believed to be involved in using relevant information supplied through connections with the amygdala, sensory cortices, hippocampus, and other regions to regulate behavioral and visceral

responses to environmental stimuli (Flynn et al.). The *medial* prefrontal cortex, including the orbitofrontal cortex and the anterior cingulate cortex (ACC), is thought to play a role in emotion-related decision-making and emotional self-regulation (Phan et al., 2004). It was found to be activated in 50% of studies examining emotional processing in humans (Phan et al.). Therefore, this may be a common region to all different emotions, including fear (Phan et al.). It has been hypothesized that the mPFC is involved in the cognitive aspects of emotional experience, including attention, appraisal and awareness of the emotion (Phan et al.). The dorsolateral PFC is thought to engage working memory processes to prepare and select responses to the threatening stimuli (Flynn et al.). The ACC is thought to be involved in regulating attention to environmental stimuli for the purpose of cognitive and emotional processing (Phan et al.). The prefrontal cortex in general may serve as a "top-down" modulator of the emotional responses generated by the amygdala.

The ventral medial, dorsomedial and dorsolateral prefrontal cortices have all been shown to activate during extinction training of a conditioned stimulus (Milad et al., 2006). Thickness of the medial OFC region has also been shown to correlate with level of extinction retention in humans (Rauch et al., 2005). Research demonstrating diminished fear extinction with prelimbic or infralimbic medial, as well as dorsomedial, prefrontal cortex lesions, support the theory that the prefrontal cortex plays an important role in the fear extinction process (Morgan & LeDoux, 1995). The vmPFC (along with the hippocampus) is also thought to play a major role in memory for fear extinction (Milad et al., 2007)

It has been demonstrated in anxiety disorders other than specific phobia (e.g. social phobia and post-traumatic stress disorder) that prefrontal cortex activity has an inverse relationship

with amygdala activity (Milad et al., 2006). Additionally, when tasks require increased cognitive effort (e.g. appraising stimulus for personal relatedness), amygdala activations decrease as PFC and ACC activations increase (Phan et al., 2004). These findings, along with results from single-cell recording studies in animals (discussed previously), can be viewed as evidence for the regulatory role of the PFC in the emotional processing network (Quirk et al., 2003; Rosenkranz, Moore, & Grace, 2003).

Although animal research on DCS and fear extinction has focused primarily on DCS effects through the amygdala and hippocampus, recent research has provided evidence that DCS also influences neural events and behavior through the mPFC (Fujihira et al., 2007; Murai et al., 2007).

Insula. The insula is closely connected to both the amygdala and the ACC and is thought to relay information regarding interoceptive and internal somatic sensations (Flynn et al., 1999). It may be involved in integrating this information with cues provided about the external environment. The insula is also known to be involved in the modulation of autonomic functions, such as cardiovascular and pulmonary activity and has been theorized to play a role in fear conditioning (Flynn et al). This region has not received as much attention as the amygdala and other areas of the PFC and therefore its exact role in emotional processing is not understood. However, current theories postulate that the insula is involved in the prediction or anticipation of body states (i.e. in response to feared stimuli) as well as the integration of somatic information and bodily states (Paulus & Stein, 2006).

Hippocampus. The hippocampus, which has been conceived as the central region associated with learning and memory, has been implicated in fear conditioning and extinction

as well. Because fear extinction is most often viewed as the learning of a new association to compete with an old association, it would be expected that the hippocampus would be involved in the process. Animal research has added much to the understanding of the hippocampi role in fear extinction and learning by demonstrating that it is necessary for recall of contextual cues related to conditioning/extinction, as well as for the normal acquisition of fear extinction (Corcoran et al., 2005; Xavier, Stein, & Bueno, 1990). It is thought to modulate the response of the amygdala to phobic or feared stimuli through neural connections with both the amygdala and the prefrontal cortex.

Animal studies have shown that DCS can enhance synaptic plasticity in the hippocampus (Billard & Rouaud, 2007; Yaka et al., 2007). Additionally, direct infusion of DCS into the hippocampus has been shown to influence learning and behavior (Ohno & Watanabe, 1998; Rouaud & Billard, 2003; Yamamoto et al., in press).

Neural Network Model of Fear Extinction. A neural circuitry responsible for fear extinction has been proposed by several researchers (Cannistraro & Rauch, 2001; Maren & Quirk, 2004; Milad et al., 2006; Myers & Davis, 2002). This theorized network primarily involves regions of the prefrontal cortex and amygdala, but also includes the insula, hippocampus, and sensory cortices. Pictoral representation of this network is displayed in Figure 1. It is hypothesized that fear extinction involves an inhibitory or regulatory power of the mPFC and the ACC over the amygdala (Maren & Quirk, 2004; Myers & Davis, 2002). This could be viewed as a mechanism through which cognitive processes are used to inhibit a conditioned response to the old association in order to allow for the establishment of a new association. Through connections between these structures and the hippocampus, new learning takes place and

contextual cues are attached to the newly learned association (Cannistraro & Rauch). The insula has not frequently been included in these theoretical models of fear extinction because its role has been supported primarily through human imaging studies and not through animal research. However, because it is thought to play an important role in the integration of information and modulation of autonomic functions, it is included in the model presented. Neverthless, because so much is unknown about this region, the direct relationship between this region and the others is not specified.

Phobia and Cognition

Neurocognitive function has become an important focus of anxiety-related research within the past decade. However, this research has concentrated primarily on obsessive-compulsive disorder (Airaksinen, Larsson, & Forsell, 2005; Dirson, Bouvard, Cottraux, & Martin, 1995; Purcell, Maruff, Kyrios, & Pantelis, 1998; Savage et al., 1996, 1999, 2000), with only a few studies published regarding panic disorder and/or social phobia (Asmundson, Stein, Larsen, & Walker, 1995; Cohen et al., 1996; Lucas, Telch, & Bigler, 1991; Purcell et al., 1998). There has been very little research examining cognitive functioning of patients with specific phobias (Castaneda et a., 2008). When this clinical group is included, it is usually to serve as a control group for the investigation of cognitive function in other anxiety disorders (Leplow, Murphy, & Nutzinger, 2002). When cognitive functioning of specific phobia patients has been compared to normal controls, no deficits have been found (Airaksinen et al., 2005). This includes measures of general intellectual functioning, verbal memory, non-verbal memory, verbal fluency, and executive functioning (Airaksinen et al.; Leplow et al.). However,

it should be recognized that thorough characterization of neuropsychological functioning in phobic individuals has not been conducted.

There is some evidence that when phobic stimuli are involved within cognitive tasks, the performance of phobic patients is negatively affected. For example, when spider-related words are included in a Stroop color naming task, spider phobic patients are slower in color naming than non-phobic controls (Thorpe & Salkovskis, 1997). It has also been reported that spider phobic patients are impaired in the recall of anxiety-related words as compared to words relating to stimulus features of spiders (Watts & Coyle, 1993). However, there have been other studies reporting *no* differences between phobic patients and non-phobic controls regarding stimulus recall or Stroop performance involving phobic-related stimuli (Kindt, Bierman, & Brosschot, 1997; Kulas, Conger, & Smolin, 2003; Sawchuk, Meunier, Lohr, & Westendorf, 2002; Thorpe & Salkovskis, 2000).

D-cycloserine and Cognition

Animal research has repeatedly demonstrated that DCS can enhance processes of learning and memory in rats and various other animals (monkeys, rabbits, etc.). These effects have been demonstrated in both normal, healthy animals (Anderson, Lindberg, & Myhrer, 2002; Land & Riccio, 1999; Lelong, Dauphin, & Boulouard, 2001; Matsuoka & Aigner, 1996; Monahan et al., 1989; Thompson & Disterhoft, 1997), as well those with chemically-induced (Kishi, Ohno, & Watanabe, 1998; Meyer, Knox, Purwin, Spangler, & Ingram, 1998; Schneider, Tinker, Van Velson, & Giardiniere, 2000) or lesion-induced (Reikkinen, Ikonen, & Riekkinen, 1998a and 1998b) cognitive deficits. Acute DCS administration appears to increase consolidation and maintenance of spatial memory (e.g. water maze task; Meyer et al., 1998;

Riekkinen et al., 1998b), non-spatial memory (e.g. simultaneous brightness discrimination test, eye-blink conditioning task; Anderson et al., 2002; Strømme Johannssen & Nyhrer, 2002; Thompson & Disterhoft, 1997), and visual recognition memory (Matsuoka & Aigner, 1996). This cognitive enhancement is thought to be mediated primarily through actions within the hippocampus. This theory is supported by findings that DCS fails to alleviate induced cognitive deficits in rats when the hippocampus is inactivated (Riekkinen, Ikonen, & Riekkinen, 1999). Additionally, Rouaud & Billard (2003) found DCS to facilitate long term potentiation and synaptic plasticity within the hippocampus of rats, providing a possible mechanism for memory consolidation and maintenance. Although animal research would suggest that DCS can enhance cognitive functioning across various tasks and domains, one cannot assume that this will necessarily hold true in human populations. Cognitive functioning in humans has evolved to a much higher, abstract level than is found in most animals and often involves verbal information. Cognitive tasks used in animals, by necessity, all involve some type of reward aspect (i.e. food) in order to motivate the animal to complete tasks. In human studies, we are able to verbally explain the rules of a task and we often rely on subject's intrinsic motivation or their feelings of obligation (i.e. to research or the investigator) to convince them to complete the task to the best of their ability. Therefore, studies concerning the effects of *acute* DCS on human cognitive functioning (with and without the aspect of reward) are needed to elucidate the findings from animal research.

In human research, there have been some clinical studies reporting *prolonged, daily* DCS administration to enhance cognitive performance of Alzheimer's disease patients (Schwartz, Hashtroudi, Herting, Schwartz, & Deutsch, 1996; Tsai, Falk, Gunther, & Coyle, 1999).

Increased cognitive functioning of Alzheimer's patients, as assessed by the Alzheimer's Disease Assessment Scale (ADAS), which includes measures of language ability, memory, and orientation to time and place, has been reported in response to 50 mg/day and 100 mg/day doses, but not with 15 mg/day doses (Tsai, Falk, & Gunther, 1998; Tsai et al., 1999). Randolph et al. (1994) found no significant clinical benefit for one week DCS administration (25, 50, 100, 175, 300, and 500 mg) on cognitive functioning as assessed by the ADAS and the Repeatable Battery for the Assessment of Dementia (RBAD). A more recent study found no clinical benefit of daily DCS administration (2, 10, 30, 100, and 200 mg/day) on cognitive functioning as assessed by the Clinical Global Impression (CGI) scale, for which the clinician provides ratings on severity of illness, global improvement, and efficacy (Laake & Oeksengaard, 2002). Therefore, it appears that while DCS *may* produce increased cognitive functioning with Alzheimer's patients, results are inconsistent.

There have also been reports that repeated DCS administration (50 mg/day) can have a beneficial effect on the cognitive functioning of schizophrenia patients, as measured by Sternberg's Item Recognition paradigm, a continuous-performance, choice-reaction-time task (Goff, Tsai, Monoach, & Coyle, 1995). However, attempts to replicate this finding have failed, despite additional (though inconsistent) reports of decreased negative symptoms in schizophrenic patients with DCS administration (Duncan et al., 2004; Goff et al., 1996; Goff et al., 2005; Goff, Henderson, Evins, & Amico, 1999).

There has been one study investigating the effects of DCS administration on the cognitive functioning of anxiety patients. Heresco-Levy et al. (2002) examined the effects of repeated DCS administration on emotional and cognitive symptoms experienced by patients with post-

traumatic stress disorder (PTSD). These subjects were given either 25 mg DCS or placebo twice daily for a period of 4 weeks. After this period, subjects underwent a 2-week "washout" period followed by a 4-week crossover treatment period. A battery of psychological and neuropsychological tests was administered bi-weekly throughout the study. Although DCS treatment was not associated with any clinically significant improvement in the emotional/psychological symptoms of PTSD, it was associated with improvements on the Wisconsin Card Sorting Test, a measure of executive function (Heresco-Levy et al., 2002).

Discrepancies in study results regarding cognitive enhancement of DCS could be due to a number of factors. It is possible that differences in outcome measures could have different levels of sensitivity to the effects of DCS. For example, the Clinical Global Impression Scale that is produced from clinician reports (Laake & Oeksengaard, 2002) may not have been as sensitive as an implicit verbal memory task (Schwartz et al., 1996). Additionally, to the author's knowledge, systematic investigation of optimal dosage for cognitive enhancement has not been conducted. Therefore, the amount and frequency of dosage may have affected the results. However, this is unlikely considering that consistent results were not even reported for studies including similar dosing schedules (Randolph et al., 1994; Tsai et al., 1998; Tsai et al., 1999). Results from animal studies with DCS may provide some insight regarding the inconsistency of results in these human studies. Every animal study reporting beneficial cognitive effects of DCS has done so using acute administration. That is, they have all measured some aspect of functioning or behavior within 30 minutes of DCS administration—not after prolonged, daily administration, as was used in the human studies.

It is possible that prolonged exposure to DCS may eliminate the enhancement properties of the drug. In fact, there have been animal studies suggesting this to be so. It has been shown that if mice are pre-exposed to 3mg/kg DCS twice a day for 15 days prior to maze training, there is no enhancement of the learning process (Quartermain, Mower, Rafferty, Herting, & Lanthorn, 1994). Even when researchers increased the dose of the DCS administered on the day of training, the beneficial effects were not reinstated. Another study demonstrated the same type of effect in relation to fear extinction. Rats were injected with 0, 1, or 5 dosages of DCS within a 10-day period (administered every other day). After this 10-day period, rats were lead through a fear conditioning paradigm. The day following fear conditioning, rats underwent a fear extinction paradigm augmented with DCS. The performance of rats given 5 previous injections of DCS was equal to rats provided saline prior to extinction. The rats with less pre-exposure to DCS exhibited the expected enhancement of fear extinction (Ledgerwood et al., 2003). Further analysis within this same study demonstrated that DCS was still able to enhance fear extinction if a 4-week interval was placed between DCS pre-exposures and the extinction paradigm. This research would suggest that prolonged administration of DCS does not have the cognitive or fear extinction enhancement properties that acute administration appears to have. If this is true for humans as well as for animals, it would eliminate any rationale for the prolonged, daily administration of DCS to treat cognitive impairment.

There has been only one study, to the author's knowledge, that has examined the cognitive effects of *acute* DCS administration. This study (Bailey et al., 2007) compared subjects treated with 50 mg DCS or placebo (90 minutes prior to testing) during either a high-anxiety (induced by Co2 inhalation) or low-anxiety (air inhalation) state on the Manikin task.

The Manikin task is a visuospatial manipulation task. For the low-anxiety groups, those treated with DCS performed better on this task (as measured by number of correct responses) and reported the task to be easier (on a self-report measure) than did the placebo-treated group. The authors interpreted this to show that DCS enhances the process of learning during low-anxiety states. However, the use of the Manikin task as a measure of learning has not been validated. Additionally, increased performance at several points during the task does not represent enhanced learning. This would be better tested through a time X group interaction analysis to examine whether the DCS group increased their performance throughout the task more than the other group. (For the study discussed, this was not the case.) Therefore, although this study suggests that DCS may modulate cognitive functioning, the results are not conclusive. Further research examining the effects of a*cute* DCS administration on cognitive functioning is needed to elucidate findings from animal research and to provide guidance for the future clinical applications of DCS.

Pharmacokinetics of D-cycloserine

DCS readily penetrates the blood-brain barrier and acts on the strychnine insensitive glycine-recognition sites of the NMDA subtype of glutamate receptors (Richardson, Ledgerwood, & Cranney, 2004). The intrinsic activity of DCS is 40 – 86% depending on the experimental paradigm used in the study. DCS is considered a partial agonist for the NR1/NR2A and NR1/NR2B heteromers. It is considered a *partial* agonist because it acts as an *agonist* in the presence of low glycine concentrations and as an *antagonist* in the presence of high glycine concentrations (Danysz & Parsons, 1998).

DCS not only has varying agonist/anatagonist properties depending on endogenous glycine levels, but also has varying properties depending upon the *dose* of DCS itself. DCS seems to act as an agonist at smaller doses and an antagonist at higher doses. This effect is most likely due to agonistic actions at NR1/NR2C receptors at lower doses and inhibition of NR1/NR2A and NR1/NR2B receptors at high doses (Danysz & Parsons, 1998).

The endogenous agent, D-serine has been found to have high concentrations in the cerebral cortex, hippocampus, striatum, and limbic system and low concentrations within the diencephalons, cerebellum, and pons-medulla in mammals. This work has been conducted primarily with mice, but similar results have been found in human and other primates. These results also correlate with the distribution of glutamate and glycine NMDA receptor sites (Nishikawa, 2005).

With a single oral dose of 250 mg DCS, peak CSF concentrations (12.8 mg/ml) are reached two hours after administration and have a plasma half-life of approximately nine hours (Nair, Epstein, Baron, & Mulinos, 1956; Peloquin, 1991). DCS has been used for more than 30 years as an antibiotic for tuberculosis, for which it has been prescribed at daily doses ranging from 500-2000 mg. Being a partial NMDA agonist, DCS does not appear to lead to excitotoxicity like full NMDA agonists (Heresco-Levy et al., 2002). In studies reporting administration of 50-500 mg DCS acutely or with treatment duration of less than one week, no clear drug-related side effects have been reported (Hofmann et al., 2006a; Kim et al., 2000; Kushner et al., 2007; Randolph et al., 1994; Ressler et al., 2004; Storch et al., 2007; van Berckel et al., 1998; Wilhelm et al., 2008). However, the following side effects of daily, prolonged administration of DCS (primarily with doses greater than 500 mg) have been

reported: tremor, hallucinations, delusions, catatonic reactions, clinical depression, exaggerated reflexes, speech difficulties (dysarthria), and slight paralysis (Cascella, Macciardi, Cavallini, & Smeraldi, 1994; Mandell & Sande, 1990; Storey & McLean, 1957). Additionally, there is some indication that epilepsy patients may be at higher risk of developing side effects from repeated administration with DCS, particularly if consumed concurrently with ethanol (Wlaz, Baron, & Losher, 1994). The occurrence of the symptoms listed has been rare, and none have occurred in response to a single dose of DCS, as used in the current study.

It is important to note that the effect of DCS administration on overall hemodynamic response has not been directly examined in human subjects (and is not measured in the current study). However, a recent study with primates provides evidence that DCS does not significant influence overall cardiovascular activity (i.e. body temperature, systolic and diastolic blood pressure; Tsukada et al., 1998).

Chapter 2: Method

Subjects

Pre-study Affective Ratings for Stimulus Validation

Prior to initiation of the proposed project, 10 spider phobic patients were recruited to provide affective ratings/validation of visual stimuli to be used during the fMRI symptom provocation paradigm. Eighty pictures of spiders and 20 pictures of butterflies, obtained from validated databases, such as the International Affective Picture System (IAPS) (Lang, Bradley, & Cuthbert, 1999), as well as from internet and other published sources, were included. Subjects were asked to rate each picture according to "arousal" using the Self-Assessment Manikin (SAM). The SAM was utilized for collection of normative data regarding the IAPS (Lang et al., 1999) and is a nonverbal method to quantify subjective feelings states. The version of the SAM that was used in the current study consisted of five icons defining a 9-point scale for arousal (1=extremely aroused, 9=extremely calm).

Subjects recruited for this validation study were not included in the entire study protocol. They were recruited in the same manner as the rest of the study subjects (described below) and screened using the Spider Phobia Questionnaire (SPQ) and eligibility survey. This screening process required approximately 15-20 minutes for completion. Those who met criteria were scheduled for an appointment at either the Kansas City Center for Anxiety Treatment (KCCAT) or Hoglund Brain Imaging Center (HBIC). At this appointment, they were asked to provide written consent. After providing affective ratings for the potential stimuli, these subjects' involvement in the study was complete. Completion of pre-study affective ratings required approximately 30 minutes of each subject's time.

There were 3 male and 7 female raters included in this validation study. The mean age was 26.50 (SD=4.50) and the mean years of education was 16.60 (SD=1.90). The average SPQ raw score for this group was 19.20 (SD=4.69). The average rating for all 80 spider pictures included was 3.90 (SD=1.74). The mean rating for the 15 butterfly pictures was 7.65 (SD=.45). The 60 spider pictures rated as most arousing by these subjects were included for use in the fMRI symptom provocation paradigm. The average arousal rating for these 60 pictures was 2.91 (SD=.45). The difference between ratings for the spider and butterfly pictures was significant ($t(73) = 36.65$, $p<.001$).

Recruitment

Throughout the one year of the present study, 54 subjects (27 phobic, 27 non-phobic healthy controls) were recruited from 1) advertisements on the KUMC and KU campuses, 2) advertisements in online community newspapers and publications (pitch.com and craigslist.com), and 3) the University of Kansas (KU) undergraduate Introductory Psychology research subject pool. To aid in recruitment, subjects were provided $50 compensation for the fMRI scanning session. Additionally, subjects were provided the opportunity to receive a black and white, printed picture of their brain, obtained through MRI.

Potential subjects within the KU-Lawrence undergraduate research pool were provided the opportunity to complete several questions from the SPQ as part of the web-based, mass pre-screening process directed by the Department of Psychology. Subjects who were identified as having symptoms indicative of spider phobia were contacted by email or phone to complete the screening questionnaire. Those identified as possible control subjects were contacted in the same manner. Potential subjects originating from sources other than the undergraduate research

pool were instructed to call or email research personnel at Hoglund Brain Imaging Center (HBIC). When a potential subject was contacted, pre-screening was conducted utilizing an eligibility survey and the SPQ. Those identified as potential subjects were scheduled for clinical assessment at KCCAT.

Consent

At the initial session (the clinical assessment session), written informed consent was obtained from each subject prior to beginning assessment. Subjects were informed that they could withdraw from the study at any time by contacting research personnel. If consent was withdrawn, participation in the study was terminated and no additional data was collected or used for analysis. However, subjects were unable to collect the $50 compensation if they failed to complete the fMRI session.

Inclusion/Exclusion Criteria

All subjects were right-handed adults between 18 and 55 years of age. Subjects in the phobic group scored at least 16 on the SPQ (which is 3.5 standard deviations above the mean for non-phobic populations) and met diagnostic criteria for spider phobia, as defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2000). Subjects included in the control group scored a 2 or below on the SPQ (which is.5 standard deviations below the mean reported for non-phobic populations). All female subjects had pregnancy ruled out via urine pregnancy (beta-HCG) test administered immediately prior to medication administration. This pregnancy test was administered by nursing staff at the General Clinical Research Center (GCRC) at KUMC. Additionally, subjects with medical conditions unsuitable for MR scanning were not included. This required

exclusion of individuals who had a pacemaker, vascular clips, or other internal metal. Because of contraindications with DCS treatment, subjects with a history of epilepsy or seizures of any etiology were also excluded. Because of increased risk of allergic reaction to the tarantula in the behavioral avoidance test, individuals reporting diagnosis of asthma or an allergy to bee stings or spider bites were excluded. Additionally, subjects reporting present or past diagnosis of a developmental disorder, neurological disorder, or head injury (with positive loss of consciousness and hospitalization) were not included. Those reporting past substance dependence, including all types of alcohol, elicit drugs, or medications, but excluding caffeine (as measured through ADIS interview) were also excluded. Spider phobic subjects reporting psychotic symptoms, major depression, dysthymia, substance abuse or dependence (excluding caffeine), claustrophobia, or any other Axis I psychopathology as defined by the DSM-IV and measured by the ADIS (other than phobia) were excluded from the study. Subjects recruited for the non-phobic control group were also excluded if they had any Axis I psychopathology as defined by the DSM-IV (American Psychiatric Association). Subjects in both groups were off all psychotropic medications for at least 6 months and had no history of treatment for anxiety or depressive disorders.

Privacy and Confidentiality

The intial assessment and all behavioral and cognitive assessments were completed in a private room with only the examiner present. If there was a reason for additional research staff to be present or to observe such assessments (e.g. for research personnel training), the subject was asked for their verbal consent. The MRI control room and scanner are located in a private area. The door to the control room was locked during

scanning to prevent individuals not involved in the study from entering. Only research personnel and MRI technicians were allowed within the control and scanning room when the subject was being scanned. Data and safety monitoring was conducted by research personnel at HBIC. Identification numbers were assigned to each subject at the time of the initial assessment. Data used for analysis were de-identified and accessible to research personnel only. The staff of the GCRC had access to information regarding demographic information, identification number, and group assignment of each subject, as well as data collected by the GCRC nurses (i.e. blood pressure, pulse, heigh, weight, and demographic information).

Procedure

Initial Assessment

Following written informed consent, clinical information was collected by a trained clinician (Robin Aupperle, M.A.) under the supervision of Lisa Hale, Ph.D. at KCCAT. Assessment included the Anxiety Disorders Interview Schedule (ADIS) for DSM-IV (Brown, DiNardo, & Barlow, 1994), the Weschsler Abbreviate Scale of Intelligence (WASI), a behavioral approach/avoidance task (BAT), and the Beck Depression Inventory, all described below. The initial assessment required approximately 1.5 to 2 hours for completion.

Anxiety Disorders Interview Schedule (ADIS) for DSM-IV. In addition to anxiety disorders, the ADIS provides modules for a wide range of psychiatric symptoms, including depression (MDD and dysthymia), psychosis, mania/hypomania, and substance abuse (Brown et al., 1994). The ADIS was used to diagnose spider phobia as well as to screen for comorbid diagnoses. Within the non-phobic control group, it was used to screen for symptoms of Axis I

psychopathology. Test-retest reliability of the ADIS was reported average to high for the following disorders when used to diagnose individuals according to the DSM-III-R: simple phobia, social phobia, obsessive-compulsive disorder, and panic disorder (Di Nardo, Moras, Barlow, Rapee, & Brown, 1993). Inter-rater reliability of the ADIS has been reported as high for both anxiety disorders and affective disorders when used to diagnose according to both the DSM-III and the DSM IV (Brown, DiNardo, Lehman, & Campbell, 2001; Di Nardo, O'Brien, Barlow, Waddell, & Blanchard, 1983). Administration time of the ADIS for an experienced examiner has been estimated at approximately 90 minutes. However, because the populations included in the current study were relatively free of psychiatric symptoms, this time was shortened significantly to approximately 45 minutes.

Spider Questionnaire (SPQ). The Spider Questionnaire was an additional tool used to diagnose (in the phobic group) and screen for (in the non-phobic group) spider phobia (Klorman, Hastings, Weerts, Melamed, & Lang, 1974). Norms for non-phobic and phobic college populations was provided by Fredrikson (1983). The mean and standard deviation for non-phobic subjects was 3.8 (3.42), while that reported for phobic subjects was 23.76 (3.8). For the current study, SPQ scores of subjects in the control group were all .5 standard deviations below the non-phobic mean (≤ 2). All scores for subjects in the phobic group were 3.5 standard deviations above the non-phobic mean (≥ 16). This instrument has been shown to have the ability to discriminate between phobic and non-phobic individuals and has been reported to have high internal consistency and test-retest correlations (Fredrikson, 1983; Johnsen & Hugdahl, 1990; Muris & Merckelbach, 1996). Additionally, it appears to be

sensitive to the effects of behavioral therapy (Muris & Merckelbach). Administration time ranged between 5-10 minutes.

Beck Depression Inventory II (BDI-II). The BDI-II is a 21-item, self-report measure and one of the most widely used instruments to assess severity of depressive symptoms (Beck, Steer, &Brown, 1996b). Subjects choose one of four statements relating to each item that best describes how they have felt over the previous two weeks. As an example, the first item is "Sadness" and the options are: 0="I do not feel sad", 1="I feel sad much of the time", 2="I am sad all of the time"; and 3="I am so sad or unhappy that I can't stand it". A total score is obtained by summing the responses to the 21 items and therefore, the range of possible scores is 0 – 63. Scores below 4 are considered below normal for healthy, non-depressed controls, 5 – 9 is considered normal (subclinical), 10 – 18 is considered mild to moderate depression, 19 – 29 is considered moderate to severe depression, 30 – 63 is considered severe depression. All subjects in the current study scored in the 'normal' or 'below normal' range. The BDI-II has been reported to have adequate internal consistency (α=.91) and test-retest reliability (r=.93), as well as construct validity as compared with the Hamilton Depression Rating Scale (r=.71; Beck, Steer, Ball, & Ranieri, 1996a; Beck et al., 1996b). This measure required 5 – 10 minutes to complete.

Behavioral Avoidance Task (BAT). Behavioral approach/avoidance tasks are used to measure levels of pathological avoidance in anxiety disorders, and are commonly implemented in the assessment of specific phobias, including spider phobia (Hersen & Bellack, 1988; McLean & Woody, 2001; Ost, 1989). For the BAT, subjects were seated in a comfortable chair at a long conference table in a room alone with the examiner. A movable box of plexiglass,

containing a live spider was placed at the far end of the table. The spider was a Chilean rose hair tarantula, chosen for its docile nature. Risks associated with handling this type of tarantula and the safety precautions taken to minimize this risk are included in Appendix C. Subjects were asked to hold a string connected to the box. They were asked to pull on the string and bring the box to the point in which they began to feel uncomfortable. It was emphasized that the subjects were not to force themselves or make themselves feel excessively uncomfortable. Performance on this test was measured by a 13-point scale: *0* = distance between subject and spider more than 8 feet, *1* = distance between 7 and 8 feet; *2* = distance between 6 and 7 feet, *3* = distance between 5 and 6 feet, *4* = distance between 4 and 5 feet, *5* = distance between 3 and 4 feet, *6* = distance between 2 and 3 feet, *7* = distance between 1 and 2 feet, *8* = distance less than 1 foot, *9* = subject's hand on box, lid is not open, *10* = lid open, *11* = lid open, hand reaching into box, *12* = holding spider in hand. Subjects were also asked to estimate their anxiety level on a SUDS scale (subjective units of distress; scale of 0 - 8) at the point in which they terminated the task. The method and scoring procedure for this task is based upon that described by Merckelbach, Jong, & Arntz (1991). The BAT took approximately 2 - 3 minutes to administer.

Wechsler Adult Intelligence Scale (WAIS) Vocabulary and Matrix Reasoning Subtests. The WAIS is "an individually administered clinical instrument for assessing the intellectual ability of adults aged 16 through 89" (Wechsler, 1997). It consists of several subtests that measure different aspects of the construct intelligence. When the complete battery of tests is used, three IQ scores (Verbal, Performance, and Full Scale) and four Index scores (Verbal Comprehension, Perceptual Organization, Working Memory, and Processing Speed) can be

obtained. However, for the purposes of this study, only the Vocabulary and Matrix Reasoning subtests were administered. These tests were used as a quick measure of both verbal and performance intelligence, to ensure that the various groups included in the study did not differ on intellectual functioning. The vocabulary test is meant to measure expressive and receptive vocabulary. It consists of 33 orally and visually presented words that the subject defines orally. Each item is scored on a three point scale (0=obviously wrong, 1=correct, but shows poverty of content, 2=shows good understanding of the word). The individual item scores are summed to obtain a total raw score, which can then be converted to a scaled score using normative data (based on age) provided in the manual (Wechsler, 1997). The matrix reasoning test is meant to measure visual information processing and abstract reasoning skills. It consists of 26 items, each of which is an incomplete gridded pattern that the subject completes by selecting one of five possible choices. The number of correct responses is summed for a total raw score. This score can be converted to a scaled score using normative data (based on age) provided in the manual (Wechsler, 1997). Administration of these two subtests in the current study was approximately 20 - 30 minutes.

fMRI Scanning and Neuropsychological Measures

Those who still met inclusion criteria after the initial assessment were scheduled for a study session that included DCS/placebo administration, fMRI scanning, and neuropsychological testing. During fMRI, a block-design, symptom provocation paradigm was utilized. The following assessment tools were utilized in the neuropsychological testing battery: 1) Rey-Osterrieth Complex Figure Test (RCFT), 2) Wisconsin Card Sorting Test (WCST), 3) Iowa Gambling Test, and 4) Wechsler Memory Scale III (WMS-III)). Completion

of all procedures involved at this one time point required approximately 5 to 6 hours. A more detailed description of the risks and benefits of the current study, as well as the data and safety monitoring plan, were prepared for the institutional review board (IRB) human subjects committee (HSC) application, and are included in Appendix A.

Medication. The study conducted by Ressler and colleagues (2004) demonstrated that both 50 mg and 500 mg doses of DCS were effective in increasing response to ERP treatment. There was some indication that the 500 mg dose was associated with less beneficial results. Clinical studies currently being conducted and published using DCS-supplemented exposure therapy are using 50-100 mg doses (Guastella et al., 2008; Kushner et al., 2007; Wilhelm et al., 2008; and information provided through consultation with Michael Davis, Ph.D.; Sabine Wilhelm, Ph.D,, and Stefan Hoffman, Ph.D.). However, D'Souza et al. (2000) was able to detect only trace levels of DCS in cerebrospinal fluid (CSF) after acute oral administration of a 50 mg dose. Research has shown that with a single oral dose of 250 mg DCS, CSF levels reach peak concentrations (12.8 mg/ml) 2 hours after administration and have a plasma half-life of approximately 9 hours (Nair et al., 1991). Subjects in the present study were administered 100 mg of DCS. This dose was chosen in order to 1) remain consistent with the current clinical investigations being conducted with DCS, 2) minimize the potential for side effects and 3) optimize the effects of the medication and the availability of the medication to the central nervous system. The DCS and placebo pills were prepared by the KUMC investigational pharmacy.

An investigational new drug application (IND) was submitted to the Food and Drug Administration on March 21, 2006. The FDA concluded that the proposed study met all of the

requirements for exemption from the IND regulations and, therefore, an IND was not required to conduct this investigation. The official letter from the FDA stating they would not accept the IND application was received on March 31, 2006.

DCS and placebo pills were obtained from and prepared by the KUMC investigational pharmacy and administered by nursing staff at the KUMC General Clinical Research Center (GCRC). Randomization of subjects to receive either DCS or placebo was conducted by Sandra Hall, Ph.D. (who, at the time, held the position of Research Assistant Professor, Department of Preventive Medicine and Public Health, KUMC), who communicated the results of randomization (subject numbers and group assignment of each) to staff at the KUMC investigational pharmacy. Dr. Hall conducted separate, block randomizations (with 12 subjects per block) for the phobic and non-phobic groups, so that half of each group was randomized to receive DCS while the other half was randomized to receive placebo. Each subject therefore had a 50% chance of receiving DCS. All investigators, test administrators, and subjects were blind to group assignment until after all data for a particular "block" had been collected and scored (or preprocessed in the case of fMRI data). Additional subjects added to the study (beyond the 24 per diagnostic group) were either randomized along with the second block of subjects or assigned to a treatment group based upon the assignment of the subject they were replacing. Regardless, the investigators remained blind to all subjects' treatment group assignment until after all data had been collected and scored. Nursing staff at the GCRC monitored subjects 2-3 hours after drug administration for potential side effects. An adverse symptom checklist (ASC) was administered after the fMRI session to assess for potential side effects.

Rey-Osterrieth Complex Figure Test (RCFT). The RCFT is used to assess visuospatial construction, learning strategy, and visual memory (Spreen & Strauss, 1998). Subjects are first asked to copy a complex figure and then to reproduce that figure by memory after a 20-minute delay. The copy and memory trial are scored on accuracy (including attributed of both "accuracy" and placement), and organization of the reproduction (Deckersbach et al., 2000; Savage et al., 1999; Taylor, 1959, as cited in Spreen and Strauss, 1998). Standardized scores are calculated based on normative data provided by Meyers and Meyers (1995). High inter-rater reliability has been reported for total accuracy scores of the RCFT, though the reliability of each individual item score is more variable (Fastenau, 1996). Scores on the copy trial of the RCFT have been reported to correlate with orbitofrontal cortex volume in obsessive-compulsive disorder, and the organizational strategies required in this test are thought to be related to frontal-striatal networks (Choi et al., 2004; Savage et al., 2000). Administration time was approximately 10-15 minutes, excluding the delay (Spreen & Strauss).

Wisconsin Card Sorting Test (WCST). The WCST is thought to measure executive functioning and working memory (Spreen & Strauss, 1998). The task requires strategic planning, use of environmental feedback, and inhibition of impulsive responses. During this test, four "key" cards are set in front of the subject: the first card has one red triangle, the second has two green stars, the third has three yellow crosses, and the fourth has four blue circles. The subject is asked to take one card at a time from two, 64-card decks (with various combinations of color, form, and number) and place each card below the key card they think it best matches. They are not told how to match the cards but are told each time whether they are right or wrong. The rule for matching the cards varies by color, form, and number, though the

subject is not informed of when the rule changes. Standardized scores are calculated by the WCST-computer scoring program and based on normative data provided in the WCST-revised manual (Heaton, 2003; Heaton, Chelune, Talley, Kay, & Curtis, 1993). The scores that were analyzed for the proposed study include total items correct, total errors made, categories completed, failures to maintain set, and perseverative responses. Standardized scores can be calculated based on normative samples separated by age and education level. The WCST has been shown to be sensitive to damage to the frontal lobe and subcortical structures (Heaton et al., 1993). Activations within the frontal (specifically the dorsolateral prefrontal cortex) and temporal regions have also been shown to relate to WCST task performance (Fallgatter & Strik, 1995; Konishi et al., 1999; Ragland et al., 1998; Volz et al., 1997).

Iowa Gambling Test. The Gambling Test was developed by Bechara, Damasio, Damasio, and Anderson (1994) and is thought to measure aspects of emotion-based learning, decision-making, goal-directed behavior, and inhibitory processes. The computer version of the Gambling Test was administered for the current study. During this test, four decks of cards (A, B, C, D) are shown on a computer screen. The subject is "given" $2,000 of virtual money to start the game (which is indicated on the computer screen). For each trial, subjects are asked to choose one card from the 4 decks presented. They are told that each time they choose a card they will win money. Every so often, however, their choice is associated with a loss of money as well. The goal of the game is to win as much money as possible. Some decks are considered "bad decks" because they are higher-risk and will lead to losses over the long run. Other decks are considered "good decks" because they are lower-risk and lead to gains over the long run. The decks differ from each other in the number of trials over which the losses are distributed.

The score for this test is based on number of cards taken from each deck ("good" decks versus "bad" decks) (Bechara, Tranel, & Damasio, 2000). Scores can be calculated in this manner for each of 5 "blocks" (trials 1-20, 21-40, 41-60, 61-80, and 81-100), as well as for the entire task. Analyses for the current study focused on total score, but scores for each individual block were also calculated. Damage to the ventro-medial prefrontal cortex is associated with deficits on the Iowa Gambling Task and activity in this area has been reported to relate to individuals' reactions to the reward and punishment aspects of the task (Oya et al., 2005). Activity in the medial frontal gyrus, as measured by fMRI, has been reported to relate to risk anticipation aspects of the task (Fukui, Murai, Fukuyama, Hayashi, & Hanakawa, 2005).

Wechsler Memory Scale, 3rd edition (WMS-III) Logical Memory and Faces Subtests. The Logical Memory subtest of the WMS-III is a test of auditory/verbal memory. Two stories are read aloud to the subject, who is then asked to recall as many details from the story as they can remember. After a 25-35 minute delay, subjects are asked to again recall as many details from each story as they can. Scoring is based upon the number of details correctly recalled for both immediate (Logical Memory I [LM-I]) and delayed (Logical Memory II [LM-II) conditions. Additionally, the percent retention score is calculated to indicate the information retained from immediate to delayed recall and is based on the following formula: (LM-II total score / LM-I story total score) *100. The Faces subtest of the WMS-III is a test of visual/nonverbal memory. Pictures of faces are displayed one at a time to the subject. After this initial presentation, subjects are shown more faces, some of which were displayed before and some of which were not. The subjects are asked to indicate whether they had viewed the faces previously ('old" faces) or if they had not ("new" faces). After a 25-35 minute delay, subjects are shown another

set of faces and asked again to indicate which ones they were asked to remember and which ones are new. Scoring is based on the number of faces correctly recalled. A percent retention score is calculated in the same way as described for the Logical Memory test. Standardized scores can be obtained for both of these tests using normative data (on age and education) provided in the WMS-III manual (Wechsler, 2002). Administration time in the present study was approximately 20-30 minutes.

Adverse Symptom Checklist (ASC): The adverse symptom checklist was adapted from a version used by Dr. Eric Storch of the University of Florida in a previous investigation examining the effects of DCS on ERP for obsessive-compulsive disorder. Dr. Storch provided the checklist for use in this study. The checklist required subjects to rate how "bothersome" they found each of 27 symptoms to be at the time of assessment, using a scale of 0 (not at all) to 3 (severe). The items related to neurological, gastrointestinal, cardiovascular, musculoskeletal, and other symptoms. As part of the ASC, subjects were also asked to indicate if they believed they were given the medication (DCS) or the placebo pill.

fMRI. MRI Scanning was performed at HBIC using a 3 Tesla (3T) head-only Siemens Allegra scanner (Siemens, Erlangen, Germany) fitted with a quadrature head coil. Following automated scout image acquisition (a low-resolution image that serves as a localizer for the remaining scans) and shimming procedures performed to improve the magnetic field homogeneity, T1-weighted anatomic images were acquired with a 3D spoiled gradient echo (SPGR) sequence (repetition time/echo time [TR/TE] = 23/4 ms, flip angle = 8°, field of view [FOV] = 256 mm, matrix = 256 x 192, slice thickness = 1 mm). This scan was used for slice

localization for the functional scans, transformation into Talairach space, and coregistration with fMRI data.

Following structural scanning, two gradient echo blood oxygen level dependent (BOLD) scans were acquired. These runs included 43 contiguous coronal slices, perpendicular to the anterior commissure (AC) – posterior commissure (PC) line (TR/TE = 3000/40 ms, flip angle = 90°, FOV = 192 mm, matrix = 64 x 64, slice thickness = 3 mm, 0.5 skip, in-plane resolution = 3 x 3 mm, 130 data points). Slice acquisition was set for whole-brain coverage (though often, the very anterior part of the occipital lobe was not obtained). Visual stimuli were projected through 3D limited view goggles (Resonance Technology, Inc., Northridge, California) connected to the stimuli-generating computer program (NeuroSTIM, Neuroscan, El Paso, TX). The proposed symptom provocation fMRI paradigm was based on paradigms used for previous functional imaging research with spider phobic patients (e.g., Straube et al., 2004).

There were three condition types included in the symptom provocation paradigm:

1. Phobic Stimulus: Subjects were exposed to pictures of spiders. The pictures used as stimuli in this paradigm were those identified through the stimulus validation procedure described earlier.

2. Non-Phobic/Neutral Stimulus: Subjects were exposed to neutral stimuli, consisting of pictures of butterflies. It was possible that patients with spider phobia would experience phobic symptoms related to other small animals including snakes, rats, and even dogs. Butterflies were, therefore, chosen as the neutral stimulus because of their similarity to spiders and because of the unlikelihood that they would invoke phobic fear in spider-phobic subjects.

Butterfly stimuli were also used in the baseline condition of Paquette et al. (2003). The pictures were of similar brightness, resolution, and size to the spider pictures.

3. Low Level Baseline Comparison: Subjects were asked to focus on blurred images of similar brightness, resolution, and size to the spider and butterfly pictures. These blurred images were created by applying a Gaussian kernel to the animal pictures (so the objects were not identifiable). This method has been used to generate low-level baseline stimuli in previous studies conducted at HBIC (e.g., Holsen et al., 2005).

For the symptom provocation paradigm, subjects underwent two fMRI functional scanning runs. Each run alternated between blocks of pictures from the phobic, non-phobic, and baseline conditions. Before the first provocation run and after each of the two runs, subjects were asked to report their subjective units of distress (SUDS) on a scale of 0 - 8 to measure the intensity of induced anxiety. The order of the blocks within a run was counterbalanced within- and across-subjects in order to prevent order effects. There were 10 novel pictures presented within each block (pseudo-randomized for every scanning run). Pictures were presented on a screen for 2.5 seconds each, with an interstimulus interval (ISI) of 0.5 seconds. Subjects were asked to remember the pictures displayed during scanning and were then given a memory task immediately following fMRI. For the memory task, 30 pictures (15 spider and 15 butterfly) shown during scanning were chosen for recall and were interspersed with 20 novel distracter images (10 spider and 10 butterfly). Outside of the scanner, subjects were shown these images on a computer screen and instructed to press one key if they believed they had been shown the image in the scanner and another key if they believe they had not been shown the image. Recognition discriminability indices were calculated for this

recognition memory test using the following formula: 100*[1-((#false positives + # false negatives)/total # pictures)]. These indices were calculated for the spider pictures, the butterfly pictures, and the total (all pictures combined).

Data analysis

fMRI data were analyzed using the BrainVoyager QX statistical package (Goebel & Jansma, 2004; Brain Innovation, Maastricht, Netherlands). Several "pre-processing" steps were taken to prepare raw BOLD data for analysis. These steps include motion correction, spatial and temporal smoothing, and anatomic transformation. First, trilinear 3D motion correction used the first image of each volume as a reference to which all other images within that volume were aligned through translation and rotation, correcting for head motion along the three axes (x,y, and z). These six motion correction parameters were also detrended (linear trend removed), z-transformed, and entered as variables of no interest in the multi-study general linear model to further correct for subject motion. Motion in any run of more than 4 mm along any axis (x, y, or z) resulted in the discard of that run. Additionally, motion that was consistent with the paradigm itself and caused visible artifact in the single-study general linear model for that run also resulted in the discard of that run. Sinc-interpolated slice scan time correction was used to shift each voxel's time series to allow all scans within a volume to be analyzed as if they were acquired at the exact same time (in reality the slices are collected over the length of the TR, in this case 3 seconds). 3D spatial smoothing with a 4-mm Gaussian filter was also conducted. Spatial smoothing reduces the resolution of an image by averaging together adjacent voxels, which increases the signal-to-noise ratio. High pass filter temporal smoothing was similarly used to smooth pixels temporally throughout a time-series, reducing the

"jitteriness" of the data. Functional images were then realigned to the anatomic images obtained within each session and normalized to the BrainVoyager template image, which conforms to the space defined by the Talairach and Tournoux's (1988) stereotaxic atlas.

Brain Voyager QX software was used to conduct statistical tests of the preprocessed data to produce statistical parametric maps (SPMs; Friston et al., 1995). Multiple regression analysis with a general linear model (GLM), allowing for multiple predictors, was used to examine differences in BOLD response between the experimental conditions (e.g. spider versus butterfly conditions). Regressors representing the experimental predictors of interest, as well as the motion correction regressors representing variables of no interest, were entered into the GLM. The regressors of interest were modeled with a hemodynamic response filter, which corrects for temporal delays in the BOLD response to a scan condition. All regressors were entered into the multiple-regression analysis using a random-effects model. Contrasts between conditions of interest were assessed with *t* statistics for each voxel. The resulting statistics for each voxel were then displayed pictorially on statistical parametric maps (different colors corresponding to varying levels of significance) and overlaid on three-dimensional renderings of an averaged-group brain. Voxel values were considered significant if they exceeded a threshold of $p<.001$ and had a minimum cluster size of 3 contiguous voxels. While the whole brain was inspected for areas of significant activation, a priori regions of interest (ROIs) were specified based on results of previous work and were the focus of analyses and discussion.

Analytical Procedures by Hypotheses

Prior to testing the specific study hypotheses, demographic, clinical, and adverse symptom data were examined for differences between groups. For continuous variables,

two-way ANOVAs were used to identify main effects and interactions for diagnosis and treatment. Because of the small sample size, Fisher's exact tests (instead of chi-square) were used to identify differences in categorical variables between diagnostic and treatment groups, as well as individual groups. Additional statistical analyses conducted (including repeated measures ANOVA, paired-samples t-tests, and Pearson correlations) will be explained in the results section when relevant. Behavioral data related to the fMRI paradigms were analyzed in the same manner. Contrasts were considered significant if they met $p < .05$.

Specific Aims 1 and 2 were tested using data collected during the fMRI symptom provocation paradigm. Data from neuropsychological testing were used to test Specific Aim 3. Hypotheses specified *a priori* are included below in italics, allowing for direct connections to be made between study hypotheses and analytical procedures.

Aim 1: There will be a significant diagnosis x condition interaction in that the spider phobic group will show greater activation than the healthy control group during presentation of spider (phobic) pictures as compared to butterfly (nonphobic) pictures, in the following brain regions (bilaterally): lateral and medial amygdala, insula, medial prefrontal cortex (including the ACC and the orbitofrontal cortex[OFC]), dorsolateral prefrontal cortex (dlPFC), and the hippocampus.

Analyses for this Specific aim 1 were restricted to the untreated (placebo) phobic and healthy control subjects. This hypothesis was directly tested using a diagnosis (phobia vs. control) x condition (spiders vs. butterflies) design. Additionally, condition main effects were conducted to investigate the neural response of each group separately.

Aim 2: There will be a significant treatment x condition interaction in which the DCS phobic group will show increased activations in the medial PFC (including ACC and the OFC), dorsolateral prefrontal cortex, and the hippocampus but decreased activations in the lateral and medial amygdala and insula compared to the placebo phobic group during presentation of spider (phobic) pictures (as compared to butterfly pictures).It is hypothesized that the DCS control group will not show differences in activation compared to the placebo control group.

Specific Aim 2 hypothesis includes subjects from all four groups (phobic placebo, phobic DCS, healthy control placebo, healthy control DCS) and relates to the effects of DCS on neural activation during phobic symptom provocation. To examine DCS effects during the provocation paradigm, the direct interactions of treatment (DCS > placebo) x condition (spiders > butterflies) were conducted for 1) the control groups and 2) the phobic groups. Main effects were then conducted within each group (DCS phobic and DCS control) to investigate the neural response of each group separately. As described previously, there were numerous studies reporting the blood flow correlates of symptom provocation in specific phobia. Research has also provided evidence of regional activations that normalize after successful ERP therapy. However, because the paradigm used does not mimic an entire treatment, it was not hypothesized that DCS administration would simply cause normalization of phobic activations. The provocation paradigm more similarly mimics that of animal and human studies examining the initial steps of the fear extinction process, in which a CS is presented after the subjects have undergone CS-UCS fear conditioning. It was therefore hypothesized that DCS would enhance the effects

associated with the initial steps of fear extinction. The hypotheses regarding the influence of DCS on brain activations during phobic provocation were based primarily upon the fear extinction research and the neural circuitry model of fear extinction outlined above. *Aim 3.1: It is hypothesized that subjects in the DCS groups (phobic DCS and healthy control DCS) will obtain better scores on all cognitive measures. It is not expected that there will be differences between Diagnostic groups on cognitive performance.*

Specific Aim 3 hypothesis relates to the effect of DCS on cognitive functioning. Aim 3 was tested through statistical analysis of individual test scores using the Statistical Package for the Social Sciences (SPSS). Neuropsychological tests were scored according to their respective manuals and/or previous literature, as described in the measures section. Raw scores were the focus of analyses for this study. There were two separate scores (accuracy and organization) for each of the Rey Complex Figure Test stages (copy, immediate recall, and delayed recall); five for the Wisconsin Card Sort (categories completed, total items correct, total number of errors, failures to maintain set, and preservative responses); one for the Iowa Gambling Task (good choices – bad choices); and six for the Wechsler Memory Scale III (Logical Memory I total, Logical Memory II total, Logical Memory retention score, Faces I total, Faces II total, and Faces retention score). Two-way ANOVAs were used to examine the diagnosis (phobic versus placebo) x treatment (DCS v placebo) interaction, as well as the main effects for both diagnosis and treatment. These same methods were used to examine differences among groups in anxiety (SUDS) level during the symptom provocation paradigm. Contrasts were considered significant if they met $p < .05$. However, the fact that multiple comparisons

were made is taken into account during discussion and interpretation. Hypotheses for Aim 3 postulated in the proposed study are based upon the animal and human research regarding cognitive effects of DCS.

Chapter 3: Results

Demographics

A total of 54 subjects were enrolled in the study. Five subjects were excluded from all analyses. One withdrew from the study because of claustrophobia symptoms in the scanner; four were excluded because of scanner artifact that made all fMRI data unusable. Therefore, a total of 49 subjects (23 control, 26 phobic) were included in analyses of demographic, clinical, and neuropsychological data. However, additional subjects had to be excluded from fMRI analyses because of excessive motion or motion that was consistent with the paradigm (i.e. movement only when seeing spider pictures). As discussed in the sections below, only the subjects included in fMRI analyses were included in the analysis of behavioral data related to the fMRI paradigm.

As described in the procedures section, two-way, multi-factor analyses of variance (ANOVA) were conducted to identify main effects and interactions of diagnosis and treatment. Repeated measures ANOVA were used when relevant. Fisher's exact tests were used to compare differences between groups regarding categorical variables. The same basic statistical steps were used for demographic, clinical, adverse symptom, behavioral, and neuropsychological data. Unless otherwise mentioned, raw scores (instead of standardized scores) were used in the analyses.

The demographic data for all subjects (N = 49) are included in Table 2. Regarding age, there were no significant main effects for diagnosis or treatment ($F_s<1$), nor was there a significant interaction ($F(1,45) = 1.71$, $p=.197$). There were also no differences in years of education by diagnosis, treatment, or the diagnostic x treatment interaction (all

Fs<or=1). In total, there were 14 males and 35 females who participated. As determined by Fisher's exact test (two-tailed), there were no differences in gender makeup between treatment (p=1.00) or diagnostic (p=.149) groups, nor were there significant differences between any of the individual groups. However, it should be noted that there was a trend for the placebo phobic group to have a lower ratio of males to females than the DCS phobic group (p=.073). This was due to randomization and not under control of the investigator.

The WAIS vocabulary raw scores correspond to an average scaled score of 13.82 (SD=2.56) for placebo controls, 13.58 (SD=1.93) for DCS controls, 14.25 (SD=2.09) for placebo phobics, 12.77 (SD=1.88) for DCS phobics, and 13.58 (SD=2.12) for the group as a whole. The matrix reasoning raw scores corresponded to average scaled scores of 13.45 (SD=2.46) for the placebo controls, 13.92 (SD=2.27) for the DCS controls, 14.42 (SD=2.23) for the placebo phobics, 13.92 (SD=2.06) for the DCS phobics, and 13.94 (SD=2.21) for the group as a whole. These scores are all in the superior range. There were no significant main effects or interactions involving diagnosis or treatment for WAIS vocabulary or matrix reasoning raw scores (all Fs<1).

Overall, the demographic measures indicate that the current study population was a young, primarily female, highly educated, highly intelligent group of individuals. No group differences in demographics were identified. Therefore, these variables are not believed to confound the results discussed below.

Clinical Measures

Clinical data for all study groups are also displayed in Table 2. It should be noted that, although data were available for the SPQ and BAT for every subject (49 subjects total), the BDI was added to the study after a few of the subjects had already been tested. Therefore, the BDI data shown in Table 2 are for 45 subjects total (19 control [9 placebo, 10 DCS]; 23 phobic [13 placebo, 13 DCS). The average BDI score for all groups was less than 3.0, which is in the subclinical or "normal" range. There was no main effect for diagnosis ($F(1,41)=2.30$, $p=.1375$), or for treatment ($F(1,41)=1.88$, $p=.178$), and no significant diagnosis x treatment interaction ($F<1$) on BDI score. The mean SPQ scores for the phobic groups were similar to that reported previously for phobic populations and the scores for the control groups were even lower than that previously reported for non-phobic populations (Fredrikson, 1983; Kindt, Brosschot, & Boiten, 1999). The average BAT score for placebo controls and DCS controls indicate that most of these individuals were able to touch or hold the live tarantula. The SUDS level (on a scale of 0-8) also stayed low for the control groups during the BAT. The average BAT scores for the placebo phobics and DCS phobics indicate that most of these individuals stopped the test when the spider was over 4 feet away. They also reported a moderate level of SUDS during this test. The main effect of diagnosis was significant for the SPQ ($F(1,45)=825.95$, $p<.001$), BAT score ($F(1,45)=887.13$, $p<.001$), and the SUDS level reported during the BAT ($F(1,45)=167.70$, $p<.001$). As apparent in Table 2, these effects were caused by the phobic groups scoring higher on the SPQ and lower on the BAT, and reporting higher SUDS on the BAT. There was no significant effect for treatment (SPQ,

F(1,45)=2.31, p=.135; BAT score, F(1,45)=1.20, p=.279; SUDS, F(1,45)=.20, p=.659), nor was there a significant diagnosis x treatment interaction (SPQ, F(1,45)=2.72, p=.106; BAT score, F(1,45)=.011, p=.918; SUDS, F(1,45)=1.27, p=.266) for any of these variables.

The clinical measures indicate that depression was not a significant problem for any of the groups (or individuals) in the study. The phobia measures (SPQ and BAT) significantly differentiated the control and phobic groups. From these results (and report on the ADIS), it can be concluded that individuals in the phobic group met criteria for spider phobia while those in the control group reported and exhibited minimal fear of spiders.

Adverse Symptom Checklist (ASC)

Data for the ASC are shown in Table 3. The ASC was added after several subjects had already completed the study and was available for a total of 36 subjects (16 control [8 placebo, 8 DCS]; 20 phobic [10 placebo, 10 DCS]). On the ASC, there were a number of subjects who reported mild symptoms such as headache, drowsiness, dry mouth, and difficulty concentrating. However, there were no serious adverse events that occurred as a result of this study and no symptoms were reported that required medical attention. Subjects were also asked to indicate which pill (DCS v placebo) they thought they had been administered. These data were available for 48 of the subjects (23 control [11 placebo, 12 DCS]; 25 phobic [12 placebo, 13 DCS). Fisher's exact tests were used to compare groups on their opinion as well as the accuracy of their opinion. For ASC score, neither the main effect for treatment (F(1,32)=.30, p=.586), nor the diagnosis x treatment

interaction (F(1,32)=1.42, p=.243) was significant. However, there was a significant main effect for diagnosis (F(1,32)=7.56, p=.010), with the phobic groups reporting significantly more side effects on the ASC than the control groups. Fischer's exact tests (two-sided) revealed that the phobic groups were also more likely than the control groups to believe they had been delivered DCS (p=.009). However, it should be noted that the phobic group was *not* significantly more accurate in their guess than controls (p=.401). When comparing treatment groups, only one variable, accuracy was significantly different. The placebo groups were more accurate in their opinion than the DCS groups (p=.003). This was caused by the fact that a much greater number of subjects believed they were given placebo (N=35) than DCS (N=13).

From results of the ASC, it can be concluded that the subjects were not able to predict whether or not they were given the active medication. It can also be concluded that administration of 100 mg DCS was not associated with significant, detectable side effects.

Neuropsychological Measures

Rey-Osterrieth Complex Figure Test (RCFT)

Average raw scores on the RCFT are shown in Table 4. There were no main effects or diagnosis x treatment interactions for RCFT immediate or delayed recall accuracy (all Fs<1). There were no treatment effects on RCFT copy organizational score (F(1,44)=.15, p=.697), but there was a main effect for diagnosis (F(1,44)=5.48, p=.024), indicating that controls had higher organization scores than phobics. The diagnosis by treatment interaction was not significant for RCFT organizational score (F<1).

Iowa Gambling Test (IGT)

One subject did not complete the IGT because he reported being told the "rules" to the test in a class he had taken previously. Therefore, 48 subjects (23 control [11 placebo, 12 DCS]; 25 phobic [12 placebo, 13 DCS]) were available for analyses related to the IGT. As shown in Table 5, there was a main effect for diagnosis on the IGT total score ($F(1,44)=7.37$, $p=.009$) in which the control groups performed better than the phobic groups. Post-hoc analyses using independent student t-tests revealed this effect was due primarily to differences in blocks 3 ($t(46)=2.28$, $p=.027$), 4 ($t(46)=2.54$, $p=.014$), and 5 ($t(46)=2.92$, $p=.005$). (However, it should be noted that if a Bonferroni correction were used, only the difference in block 5 would remain significant.) There was a trend for treatment to have an effect on IGT total score ($F(1,44)=3.67$, $p=.062$). The diagnosis x treatment interaction was not significant ($F(1,44)=.85$, $p=.361$). Repeated measures ANOVA was conducted to examine if there were any main effects or interactions involving diagnosis and treatment for the change in score across IGT blocks. Across all groups, there was a linear increase in IGT block score ($F(1,44)=55.01$, $p<.001$) and this trend was affected significantly by diagnostic group ($F(1,44)=5.28$, $p=.026$), indicating that phobics had less of a linear increase in score across blocks. There were no other differences for the change in score across blocks (all $Fs<1$). The performance of phobic and control groups across the five IGT blocks is displayed in Figure 2.

Wisconsin Card Sorting Test (WCST)

One subject did not complete the WCST because he reported previously being shown how to score the WCST. Therefore, a total of 48 subjects (23 control [11 placebo,

12 DCS]; 24 phobic [13 placebo, 12 DCS]) were available for analyses related to the WCST. As shown in Table 6, all subjects performed very well on the WCST. Almost every subject completed all 6 sets (M=5.85, SD=.74). There were no significant differences in WCST performance by diagnosis or treatment, nor was there a diagnosis x treatment interaction (all Fs<1).

Wechsler Memory Scale (WMS-III)

The raw scores for the Wechsler Memory Scale (WMS-III) Logical Memory and Faces subtests are shown in Table 7. Repeated measures ANOVAs were used to investigate main effects and interactions involving diagnosis and treatment for the WMS-II immediate and delayed recall scores (separate analyses were conducted for the Logical Memory and Faces subtests). As indicated in Table 7, there were no significant main effects for diagnosis (LM, $F(1,45)=1.16$, $p=.287$; Faces, $F(1,45)<1$), treatment (LM, $F(1,45)<1$; Faces, $F(1,45)=1.09$, $p=.302$), or for the diagnosis x treatment interaction (Fs<1).

In summary, neuropsychological results suggest that the control group performed better than phobics on measures of emotional decision making (IGT) and organization of visuospatial information (RCFT). DCS did not seem to have a profound effect on neuropsychological functioning, though there was trend for DCS to negatively impact performance of controls during the emotional decision making task (IGT).

Provocation Paradigm

Three subjects were excluded from analyses for the fMRI provocation paradigm, as they exhibited motion that was consistent with the paradigm (movement only during

the spider condition). When motion is consistent with the paradigm, the motion correction steps described earlier are unable to completely correct for it (because the signal strength changes caused by motion cannot be disentangled from those caused by the paradigm itself), and artifact is visible in the GLM results (Friston et al., 1995; Hajnal, Myers, & Oatridge, 1994; Morgan et al., 2007). Therefore, there were 46 subjects included in analyses for this paradigm (23 phobic [12 placebo, 11 DCS], 23 control [11 placebo, 12 DCS]).

Behavioral Analyses

Subjective units of distress levels (SUDS; 0 – 8) were reported prior to the first provocation run (Pre SUDS) and after each of the two provocation runs (SUDS 1 and SUDS 2). Additionally, recognition discriminability indices were calculated for subjects' recognition memory performance after fMRI scanning (for spider pictures, butterfly pictures, and total recognition). These variables were compared between groups in the same manner as the demographic, clinical, and neuropsychological data (described above) and the results are displayed in Table 8. As shown, both phobic groups reported average anticipation SUDS (Pre SUDS) at approximately 3.0 whereas SUDS for the actual pictures was approximately 5.0 to 6.0 and stayed at that level for spider pictures during the second scan. Average SUDS for the control groups stayed close to 0 at all time points. Univariate ANOVA revealed there to be a significant main effect for diagnosis on anticipatory SUDS level ($F(1,42)=70.42$, $p<.001$). There was no effect for treatment and no diagnosis x treatment interaction on anticipatory SUDS ($Fs<1$). Repeated measures ANOVA revealed a significant main effect for diagnosis on SUDS levels for scan 1 and 2

($F(1,42)=671.70$, $p<.001$). There were no significant differences in SUDS levels for treatment ($F(1,42)=2.03$, $p=.162$) or for the diagnostic x treatment interaction ($F(1,42)=1.83$, $p=.183$). Results also indicated that there was no significant difference in SUDS between scan 1 and 2 ($F(1,42)<1$). This remained true when constricting analyses to the phobic group only ($F(1,21)<1$). This indicates that there was no habituation of SUDS during the scanning session.

As shown in Table 8, there was a main effect for diagnosis on recognition discriminability for spider stimuli (Spider D'; $F(1,42)=6.582$, $p=.014$), as well as on total recognition discriminability (Total D'; $F(1,42)=6.02$, $p=.018$). These differences came about because phobics performed better than controls. However, there was no effect of diagnosis on recognition of butterfly pictures (Butterfly D'; $F(1,42)=.05$, $p=.825$). Neither the treatment main effect nor the diagnosis x treatment interaction were significant for any of these recognition variables (all $Fs<1$).

As intended, the provocation paradigm seemed to elicit anxiety in the phobic but not in the control group (indicated by SUDS levels). The phobic group also exhibited better memory for spider stimuli than the control group. DCS did not seem to have an effect on subjective anxiety or memory for emotional stimuli.

fMRI analyses

For the fMRI analyses, only the specified regions of interest (and regions adjacent to those of interest) will be discussed and presented in the tables referenced. However, the full results for each contrast mentioned are included in Appendix B. The basic steps for analyses included 1) examining the direct interaction of group x condition (results

displayed in Table 9 and 10) and 2) examining main effects for spider versus butterfly within each group separately (results displayed in Table 11).

Specific aim 1. This aim related to differences between diagnostic groups in neural response to phobic symptom provocation. To test this aim, the diagnosis (phobic > control) x condition (spider > butterfly) interaction effect was examined within the placebo-treated groups. As shown in Table 9, this interaction revealed significant differences between the placebo phobic and control groups (for spiders > butterflies) in regions of interest including the right bed nucleus of the stria terminalis (BNST, extended amygdala; t(21)=4.42, x,y,z=9,-1,-2), right insula (t(21)=-4.70; x,y,z=33,-16,13), right parahippocampal gyrus (PHG; t(21)=-4.80; x,y,z=24,-25,-20), left dorsolateral prefrontal cortex (dlPFC; t(21)=-4.54; x,y,z=-48,23,31), and left putamen (t(21)=4.56; x,y,z=-24,-7,1) . It should be noted that the findings within the insula and PHG were in the opposite direction from expected—with the control subjects showing greater differences for spiders versus butterflies. To further investigate the reason for this unexpected finding, we examined the group interaction regarding spiders versus low-level baseline, as well as butterflies versus low-level baseline. These results are also displayed in Table 9. For the spider condition, phobics showed greater activations than controls in the dorsal anterior cingulate (ACC; BA24; t(21)=4.24; x,y,z=0,5,40), bilateral insula (t(21)=6.03; x,y,z=39,-10,-8), bilateral caudate (t(21)=4.48, x,y,z=-15,5,1), left putamen (t(21)=4.13; x,y,z=-24,-4,1), right BNST (t(11)=4.88, x,y,z=9,-1,-2), and bilateral frontal operculum (t(21)=5.26; x,y,z=45,14,-2). For the butterfly condition, the phobics also showed greater activations than controls in the left ACC (t(21)=4.67; x,y,z=-6,29,10), bilateral posterior cingulate

(t(21)=5.24; x,y,z=12,-16,37), bilateral insula (t(21)=5.32; x,y,z=-36,25,10), left caudate (t(21)=4.35; x,y,z=-24,8,7), right putamen (t(21)=4.75; x,y,z=15,14,1), and bilateral frontal operculum (t(21)=4.58; x,y,z=42,-4,4). These results suggest that, within the phobic group, many regions of interest were activating in response to *both* spider and butterfly conditions. This could have contributed to the limited (and somewhat inconsistent) findings from the diagnostic group interaction for spiders versus butterflies.

The main effect of condition was also examined within each of the placebo groups separately and these results are listed in Table 11. Comparing spider and butterfly conditions, the placebo phobic group showed activations that were greater for spiders versus butterflies in several of the predicted regions, including left lateral amygdala (cluster spreading into central and basomedial amygdala; t(11)=9.06; x,y,z=-24,-4, -14), right central (t(11)=4.72, x,y,z=24,-4,-11) and basomedial amygdala (t(11)=5.97; x,y,z=18,-1,-11), right BNST (t(11)=4.93; x,y,z=12,-1,-5), right insula (t(11)=5.41; x,y,z=36,2,1), right dlPFC (BA9; t(11)=7.35; x,y,z=42,5,37), and bilateral hippocampus (t(11)=6.37; x,y,z=27,-10,-14), as well as other related regions, including the right ventrolateral PFC (vlPFC; BA45/46; t(11)=8.22; x,y,z=45,32,10) , right insular claustrum (t(11)=5.71; x,y,z=30,11,1), left limitans claustrum (t(11)=6.88; x,y,z=-33,-13,-5), right ventral pallidum (t(11)=4.93; x,y,z=12,-1,-5), and left ventral putamen (t(11)=5.33; x,y,z=-30,-22,-5). Several of these activations are displayed in Figure 5. The placebo control group showed greater responses to spiders than butterflies in similar areas, including the left lateral amygdala (t(10)=5.29; x,y,z=-27,-4,-20), right preamygdalar claustrum (t(10)=5.32; x,y,z=27,-1,-11), right insula (t(10)=5.12; x,y,z=36,2,1), left OFC

(BA47; t(10)=5.37; x,y,z=-30,32,-5), left superior frontopolar area (BA10; t(10)=-5.40; x,y,z=-18,59,1), and right inferior frontal gyrus (BA45; t(10)=5.59; x,y,z=45, -1,31). Several of these activations are displayed in Figure 6. The contrast between spider and butterfly conditions resulted in increased activation for the phobic group in regions of interest that were not found for the control group. These regions included the basomedial and central amygdala, CA1 and CA3 fields of the hippocampus, and the dlPFC.

Results related to specific aim 1.1 suggest that the following brain regions activated in response to phobic stimuli in a way that was unique to the phobic group: amygdala, insula, cingulate, hippocampus, and dlPFC. Figure 3 shows average BOLD activation (% signal change) for selected regions that were significant for the spider > butterfly contrast within the placebo phobic but not the placebo control group. As shown, there is a trend for the phobic group to have greater activations than controls in the right basomedial amygdala and left hippocampus during the phobic condition, though this difference did not reach the specified level of significance. However, also shown are BOLD activations within the BNST, in which phobics exhibited increased activation to phobic stimuli, while controls showed the opposite response. This caused the diagnosis x condition interaction to be significant for this region.

Specific aim 2. This aim related to the effects of DCS on neural activations during symptom provocation. Results from the direct treatment group (DCS > placebo) x condition (spider > butterfly) interaction are displayed in Table 10 and Figures 5 and 6. The DCS phobic group had greater differential activations (spiders > butterflies) than the placebo phobic group in the following regions of interest: left dlPFC (BA9 & 46;

t(21)=4.41; x,y,z=-36,29,31), bilateral inferior frontal gyrus (t(21)=4.66; x,y,z=48,8,28), right dorsal ACC (t(21)=3.98; x,y,z=12,14,34), and bilateral insula (t(21)=6.19; x,y,z=-39,-7,10). Many of these activations are displayed in Figure 5. We knew from analyses comparing placebo phobic and placebo control groups that many regions of interest exhibited increased activation to butterfly as well as spider stimuli for the phobic group. Therefore, we also examined the effect of DCS on activations for the spider and butterfly conditions separately (as compared to the low-level baseline). The DCS phobics had greater differential activations than placebo phobics when comparing spider and low-level baseline conditions in the following regions of interest: left inferior frontal gyrus (t(21)=4.88; x,y,z=-48,23,22), and right dlPFC (t(21)=4.14; x,y,z=27,41,31). The left presubiculum showed greater differential activation for the placebo group than the DCS group (t(21)=-4.66; x,y,z=-18,-22,-14). It is important to note that, when looking at Table 10 and the table in Appendix D, almost all of the activation differences found for this contrast were greater for the *DCS-treated subjects*. There were significant differential activations between the DCS and placebo phobic groups for the butterfly versus low-level baseline condition in the left inferior frontal gyrus (t(21)=-4.75;x,y,z=-33,1,31), left dlPFC (t(21)=-3.94; x,y,z=-18,35,31), left ACC (t(21)=-5.61; x,y,z=-3,-31,34), left caudate (t(21)=-4.64; x,y,z=-24,8,7), right subiculum (t(21)=-4.34; x,y,z=24, -37,-5), left parasubiculum (t(21)=-4.00; x,y,z=-18,-13,-20), and right claustrum (t(21)=-4.21; x,y,z=30,-22,4). Almost all of the activations differences found for this contrast were greater for the *placebo-treated subjects*.

The direct Group X Condition interaction revealed significant differences between the DCS and the placebo control groups (for spiders > butterflies) in only the following two areas related to a priori regions of interest: left ventral ACC (BA 24; 6 voxels; t(21)= 3.916084, x,y,z = -6,26,4) and left caudate (t(21)=4.61; x,y,z=-9,14,10). When comparing these groups with regard to the contrast of spiders versus low-level baseline, no regions of interest showed significant differential activations, though a small cluster in the frontal operculum was significantly greater for DCS controls (t(21)=3.92; x,y,z==42,11,7). When comparing the groups with regard to the contrast of butterfly stimuli versus low-level baseline, the left insula (t(21)=3.99; x,y,z=-33,13,10), left putamen (t(21)=4.446; x,y,z=-24,2,16), left frontal operculum (t(21)=4.58; x,y,z=-36,14,16), and left caudate (t(21)=4.12; x,y,z=-18,-1,22) activated significantly more in the DCS group than the placebo control group. Because there are so few significant findings with respect to the comparison between DCS and placebo control groups, these results are only shown in Appendix B. Many of the significant differential activations for this interaction are displayed in Figure 6.

The main effects for spider versus butterfly conditions were also examined separately for each of the DCS groups (phobic and control). This allows for comparisons to be made with the results reported earlier for each of the placebo groups. The DCS phobic group showed greater activations for spiders versus butterflies in many different regions of interest, including bilateral insula (t(10)=8.03; x,y,z=-30,20,10), bilateral inferior frontal gyrus (t(10)==5.79; x,y,z=54,5,25), bilateral dlPFC (t(10)==8.00; x,y,z=-24,44,28), right OFC (t(10)=5.00;x,y,z=-33,35,-11), bilateral dorsal ACC

(t(10)=6.78;x,y,z=-3,17,31 and t(10)=8.79; x,y,z=6,2,46). These results are listed in Table 11 and many of the activations are displayed in Figure 5. In contrast to the placebo phobic group, the DCS phobic group did *not* have significant activations within the amygdala or hippocampus, though there was a cluster within the left basolateral amygdala that did not meet the cluster threshold. It is also important to note that the placebo phobic subjects did not have significant activations in the inferior frontal gyrus, OFC, or cingulate. The DCS control group showed greater activations for the spider versus butterfly condition in the following regions of interest, all left lateralized: superior rostral gyrus (t(11)=6.39; x,y,z=-6,56,4), dlPFC (t(11)=5.23; x,y,z=-42,5,43), lateral PFC (t(11)=5.60; x,y,z=-48,26,7), left lateral OFC (t(11)=5.12;x,y,z=-36, 29,-11), anterior (t(11)=6.90; x,y,z=-15,44,10) and posterior cingulate (t(11)=4.80; x,y,z=-3,-22,34). These results are listed in Table 11 and many of the activations are displayed in Figure 6. Again, it should be noted that the DCS control group did *not* show significant activations within the amygdala whereas the placebo control group did. The placebo control group, on the other hand, did not show significant activations within the dlPFC, vlPFC, superior rostral gyrus, or cingulate.

To illustrate the DCS findings, the BOLD % signal change in selected regions of interest found to be significantly different between the placebo and DCS phobic groups (spider > butterfly) are presented in Figure 4. As shown, DCS was associated with enhanced signal change within the insula and dorsal ACC (as well as areas of the PFC, not shown). Signal change for the left lateral amygdala, which was significant within the placebo- but not the DCS- phobic group, is also shown in Figure 4. As shown, the

placebo- and DCS- phobic groups exhibited similar patterns of activation in the left lateral amgydala. Based on this observation, as well as the fact that the treatment x condition interaction was not significant within the amgydala, it can be concluded that DCS did not significantly modulate activity in this region. The differences between groups are apparent pictorially in Figure 5.

In summary, results suggest that DCS may exert an influence on neural response patterns to phobic stimuli by enhancing PFC, ACC, and insula activations for both phobic and non-phobic subjects. However, this effect may be more robust when the individuals are experiencing a phobic (or emotional) response to the stimuli.

Correlational analyses. Correlational analyses were conducted within the phobic groups to examine the relationship between 1) regions of interest and both clinical and behavioral data and 2) between amygdala and prefrontal regions of interest (because of theorized inhibitory relationships). Regions of interest for each phobic group (DCS or Placebo) were those found significant for the spider > butterfly contrast within that group. Correlations were conducted using bivariate Pearson correlations. These correlational analyses were conducted for the two phobic groups (DCS and placebo) separately. Correlations were considered significant if they met $p<.05$.

Amygdala regions for the placebo phobic group included the left lateral amygdala (x,y,z=-24,-4,-14), right central amygdala (x,y,z=24,-4,-11), right basomedial amygdala (x,y,z=18,-1,-11), and right BNST (x,y,z=12,-1,-5). Prefrontal cortex regions for this group included the right vlPFC (45, 32, 10) and dlPFC (42, 5, 37). Also included in correlational analyses for the placebo phobic group was the right insula (x,y,z=36,2,1).

For the DCS phobic group, the maximum voxel of the left basolateral amygdala (BLA; x,y,z=-18,-1,-18) was used for correlational analyses. This region was significant for the spider > butterfly contrast within the DCS group, though did not meet the specified cluster threshold. Prefrontal cortex regions included the left (BA9; x,y,z=-24,44,28) and right dlPFC (BA 46, x,y,z=45,35,13), left (x,y,z=-45, 20, 22) and right inferior frontal gyrus (IFG; x,y,z=-45, 20, 22), and left (x,y,z=-33,35,-11) and right OFC (x,y,z=45,17,-5), Additional regions of interest included in correlational analyses for the DCS phobic group included two regions of the dorsal ACC (BA 24, x,y,z=6,2,46; BA 32, x,y,z=-3,17,31) and the left (x,y,z=-30,20,10) and right insula (x,y,z=36,2,4).

Within each group separately, the amygdala, insula, and prefrontal regions were examined for significant correlations with measures of phobia symptoms (SUDS during fMRI, BAT, SPQ). For the placebo phobic group, there were significant correlations between the SPQ and 1) right basomedial amygdala (r=.70, p=.011), and 2) left vlPFC (r=.60, p=.038). There was also a significant correlation between fMRI SUDS level and the left lateral amygdala (r=.65, p=.023). There were no correlations within the placebo phobic group for the BAT. In the DCS phobic group, there were significant correlations between SUDS level and activation in the right OFC (r=.63, p=.039) and between BAT score and the right IFG (r=.72, p=.012). There were no other correlations for these variables in the DCS phobic group.

Correlations were also examined between the amygdala and areas of the PFC. Coordinates for both the amygdala and PFC regions for each group are described above. In the placebo phobic group, there were *positive* correlations between the right

basomedial amygdala and both the vlPFC (r=.76, p=.004) and dlPFC (r=.59, p=.045). For the DCS phobic group, there was a significant *negative* correlation between the left BLA and the left OFC (r=-.75, p=.008).

Results from correlational analyses indicate that the right basomedial amygdala and vlPFC correlated with severity of phobia, while left lateral amygdala correlated with SUDS level, in the placebo phobic group. In the DCS phobic group, only prefrontal regions (IFG and OFC) correlated with phobia measures (BAT and SPQ). Additionally, in the DCS group, there was a *negative* correlation between the OFC and BLA, while in the placebo group, prefrontal and amygdala activations were *positively* correlated. Therefore, DCS potentially serves to strengthen the *inhibitory* connections between the PFC and the amygdala.

Chapter 4: Discussion

This study examined the effects of DCS on brain activations for both spider phobic and healthy control subjects during phobic symptom provocation. Results from this study replicate findings from previous fMRI symptom provocation studies and demonstrate that un-treated phobic subjects have unique activations to phobic stimuli (compared to non-phobic controls) in the bilateral central and basomedial amygdala, right bed nucleus of the stria terminalis (BNST; extended amygdala), bilateral hippocampus, and right lateral prefrontal cortex (PFC; dorsolateral [dlPFC] and ventrolateral [vlPFC]). Study results provide evidence that, in phobic subjects, DCS enhanced bilateral PFC (dlPFC and inferior frontal gyrus), dorsal anterior cingulate (ACC), and bilateral insula activations, and led to unique activations in the OFC, during phobic symptom provocation. For controls, DCS administration enhanced activations to spider stimuli in the ventral ACC and left caudate, and led to unique activations (compared to placebo controls) in the left lateral PFC (dlPFC and vlPFC). Results of neuropsychological testing provide evidence that specific phobia may be associated with subtle differences in cognitive functioning, including decreased performance during emotional decision-making and qualitative differences in strategic processing. There was a trend for DCS to negatively affect performance of non-phobic controls during emotional decision-making tasks. More in-depth discussion of findings from each aspect of the current study is presented below.

Clinical Measures and Adverse Symptoms

The mean SPQ scores for the phobic groups were similar to those reported previously for phobic populations (Fredrikson, 1983; Kindt, Brosschot, & Boiten, 1999).

Based on the BAT and SPQ, as well as the ADIS, it can be concluded that the subjects in the phobic group met criteria for spider phobia and that those in the control group had little, if any, fear of spiders. Although findings from the adverse symptom checklist suggest phobic individuals may be hypervigilant toward medication effects, there was no indication of side effects related to D-cycloserine (DCS). This is consistent with reports from clinical studies using similar doses of DCS (Guastella et al., 2008; Hofmann et al., 2006a; Kushner et al., 2007; Ressler et al., 2004; Storch et al., 2007; Wilhelm et al., 2008). It can be assumed that subjects were unable to determine whether they had been administered DCS or placebo. Therefore, results are most likely *not* due to altered expectations of functioning by those administered DCS.

Neuropsychological Measures: Diagnostic effects

Compared to the control group, the phobic group exhibited performance differences on various neuropsychological measures. These differences consisted of 1) decreased organizational strategy on the Rey Complex Figure Test (RCFT) and 2) decreased performance on the Iowa Gambling Test (IGT). Neuropsychological deficits in the specific phobics in the current study are surprising given the fact that no such differences have previously been identified. Only one other study (Airaksinen et al., 2005) has examined the neuropsychological performance of phobic subjects on neutral tasks that are unrelated to their phobia. In this study, no differences between phobic and non-phobic subjects were found. However, this study did not examine the qualitative way in which information was processed, nor did it include measures involving emotional decision-making.

The current neuropsychological results suggest that individuals with specific phobia may use qualitatively different strategies during nonverbal memory encoding tasks. The phobic subjects were less organized when copying the RCFT figure, but showed normal recall. This suggests that they were either using other, uncommon but equally effective, strategies to encode the information or were using compensatory mechanisms (i.e. enhanced memory for details) to maintain their performance. Decreased use of the common structural organization scheme on the RCFT has been reported for other anxiety disorder populations, primarily OCD (Savage et al., 1999; Savage et al., 2000) and OCD-related disorders (Deckersbach et al., 2000; Sherman et al., 2006). However, for these populations, the decrease in organization is usually associated with subsequent decreased recall performance. In contrast, studies examining encoding strategy in anxiety disorders other than OCD, such as PTSD (Jenkins et al., 1998; Moritz et al., 2005), have not found significant differences on RCFT organization and recall compared to non-anxious control groups.

Researchers have suggested that individuals use emotional signals during the Iowa Gambling Task to guide decision-making even when they are not explicitly aware of the reasons for those decisions (Bechara et al., 1997; Miu et al., 2008). In light of these suggestions, it is surprising that performance of phobic individuals on the IGT or other emotional decision-making tasks has not been reported previously. Decreased IGT performance has been reported in OCD, though results have been inconsistent (Cavallaro et al., 2003; Kuelz, Hohagen, & Voderholzer, 2004; Nielen et al., 2002). Panic disorder patients have been shown to perform similar to controls on the IGT (Cavedini et al.,

2002). However, decreased performance on the IGT *has* been reported for individuals with high trait anxiety (Miu et al., 2008). It has been suggested that in anxiety disorders (especially OCD), increased baseline levels of emotional activation decrease the "signal-to-noise" ratio and prevent adequate detection of subtle emotional changes (Savage, 2002). Inability to detect these emotional "hints" could contribute to impaired decision-making on tasks such as the Iowa Gambling Test. Future research should aim to test such hypotheses by examining emotional decision-making in a variety of anxiety-disordered populations.

Because the current study employed a small sample size and findings are in opposition to those of previous studies involving non-OCD subjects, these neuropsychological results should be considered preliminary. However, the results provide initial support for subtle neuropsychological differences associated with specific phobia that warrant further investigation.

Phobics also exhibited enhanced memory for spider pictures during the fMRI symptom provocation paradigm. This is consistent with a previous report of increased memory for phobic-related script information (Kindt et al., 1999). It is also in line with the generally held understanding that memory is greater for events that elicit emotional arousal (McGaugh, 2006). However, it should be noted that some studies have found no differences between phobic and non-phobic subjects' recall of phobic-related stimuli (Kulas et al., 2003; Thorpe & Salkovskis, 2000). The inconsistencies may stem from variations in the type of stimuli used as well variable task difficulty. In the current study, the encoding task (the symptom provocation paradigm) was of a long duration (12

consecutive minutes) and the recognition task was relatively difficult. Emotional arousal to the spider pictures may have enabled phobics to sustain attention throughout this extended time period, thereby increasing memory recall.

Neuropsychological Measures: Treatment effects

D-cycloserine was not associated with profound differences in neuropsychological functioning in the current study. However, there was a trend for DCS to negatively affect IGT performance. Larger studies are needed to examine the effects of DCS on emotional decision-making.

The fact that DCS failed to have an effect on memory for phobic-related stimuli was surprising considering theories postulating that DCS enhances memory consolidation related to fear extinction (Davis et al., 2006; Hofmann et al., 2006b). As previously mentioned, the sample size in the current study was relatively small for examining differences in behavioral measures. Additionally, we only measured one aspect of memory for the phobic stimuli, namely explicit recognition. DCS may influence different aspects of memory, such as consolidation of stimulus-response associations, or enhanced memory of contextual and abstract information. These alternatives will be discussed further in relation to the neuroimaging results. Further examination of the specific cognitive effects of DCS could be useful for understanding its potential in terms of enhancing ERP therapy.

Provocation paradigm: Diagnostic effects

Levels of fear (SUDS ratings) reported for spider pictures during the fMRI symptom provocation paradigm were significantly higher for the phobic than control

groups, supporting the use of this paradigm in provoking phobic symptoms. Additionally, these SUDS levels were similar to fear and arousal levels reported in other neuroimaging studies of spider phobia (Paquette et al., 2003; Schienle et al., 2005, 2007; Straube et al., 2006), supporting the generalizability of results. The lack of behavioral habituation to the spider pictures was evident by the fact that there was no change in SUDS level from scan 1 to 2. Therefore, it can be concluded that both scans were successful at provoking phobic symptoms.

Symptom provocation (as indicated through the contrast of spiders > butterflies) within the placebo phobic group was associated with activation in areas of the amygdala (including the BNST), hippocampus, and PFC. These results are consistent with previous research and a priori hypotheses. Results for the direct interaction of diagnosis (placebo phobic > placebo control) X condition (spider > butterfly) indicate that the BNST and a region within the striatum (left putamen) exhibited greater differential activation for the placebo phobic group while the right insula, right parahippocampal gyrus (PHG), and left dlPFC were greater for controls.

Although the results for the diagnosis X condition interaction only partly support a priori hypotheses, they are not surprising. Meta-analyses and reviews of neuroimaging in specific phobia *do* report consistent findings of greater activation in phobics than controls in the amygdala, insula, and PFC (Etkin & Wagner, 2007; Gottfried & Dolan, 2004; Miller et al., 2005; Stein, 2006). However, results from diagnostic group X condition interaction analyses are inconsistent across individual studies. For example, Paquette et al. (2003) reported phobics to have greater activations in only the inferior

frontal gyrus and PHG; Schienle et al. (2007) reported phobics to have greater activations in the amygdala and fusiform gyrus while controls had greater activations in the ACC, orbitofrontal cortex (OFC), and dlPFC; and Straube et al. (2006) reported the phobic group to have greater activation in the insula and ACC while controls showed increased activation in the amygdala and PHG. The fact that these studies utilized varying types of stimuli as "control" conditions (i.e. snakes, mushrooms, butterflies, household items) may have contributed to the inconsistencies.

When contrasting each of the "active" conditions (spider and butterfly) with the low-level baseline condition in the current study, it was found that the phobic group had greater responses than controls to *both* spider and butterfly stimuli (though more so to the spider stimuli). Results from the diagnosis X condition (spider > butterfly) interaction were, therefore, not reflective of regions specific to phobic symptom provocation, but those in which the phobic group activated more to the butterfly condition. The idea that specific phobia is associated with abnormal activation patterns to stimuli unrelated to the phobia (and that do not increase anxiety or distress) is an interesting topic of research. However, it was not the focus of this study. Instead, we were interested in neural activation patterns unique to the processing of phobic stimuli for individuals with specific phobia. Therefore, the unique activations of phobic subjects (compared to control subjects) for the spider > butterfly contrast will be the focus of the following discussion regarding neural correlates of phobic fear.

The regions which were activated uniquely for the placebo phobic group during symptom provocation (spider > butterfly), included right dlPFC and vlPFC, bilateral

central and basomedial amygdala (lateral amygdala was activated in both phobics and controls), right BNST (also significant for direct group interaction), bilateral hippocampus, and left putamen (also significant for direct group interaction). Left lateral and right basomedial amygdala activations were also found to significantly correlate with measures of phobia severity (as assessed by fMRI SUDS levels and the SPQ, respectively). Activation within the vlPFC also correlated significantly with SPQ score. Interestingly, there were positive correlations between the right basomedial amygdala and both the vlPFC and dlPFC.

Much of the focus in animal and human research of fear processing has been on the *medial* PFC. Lesions in this area strongly influence emotional behavior and activation of this area seems to enhance fear extinction (Herry & Garcia, 2002; Jinks & McGregor, 1997; Milad & Quirk, 2002; Quirk et al., 2003). However, lesions to the *lateral* PFC have been shown to influence emotional behavior in animals as well—increasing responses to conditioned fear (Lacroix et al., 2000). It is important to recognize that the lateral PFC seems to be especially developed in humans and may therefore have unique functions that are not completely homologous in animals. Several human imaging studies have reported lateral PFC activation during either symptom provocation in anxiety disorders or during fear processing in non-anxious controls (Dunsmoor et al., 2007; Milad et al., 2006; Paquette et al., 2003; Schienle et al., 2005). In support of these findings, the current study found right dlPFC and vlPFC activations that were unique to the phobic group (compared to the placebo control group) during exposure to phobic stimuli. Regions of the lateral PFC have been implicated in both reappraisal and distraction

methods of cognitive modulation during anticipatory anxiety (in healthy, non-anxious controls; Kalisch et al., 2005, 2006). The dlPFC in particular is believed to be involved in working memory and the selection of responses to emotional stimuli (Miller et al., 2005). Dorsolateral PFC activation has also been shown to activate for phobics that successfully control their behavioral response to symptom provocation, but not for those who experience panic symptoms (Johanson et al., 1998). The existence of lateral PFC activations in the current study may reflect attempts to control responses to phobic stimuli, thus allowing subjects to maintain focus on the task at hand and complete the fMRI scan. However, the fact that anxiety remained relatively high and PFC activation correlated *positively* with amygdala activation suggests that these attempts were at least partially unsuccessful.

The amygdala is thought to lie at the center of the brain's emotional processing system and is involved in rapid fear responses (Lang, Davis, & Ohman, 2000; Walker et al., 2003). As discussed previously, it has been shown to be involved in both fear acquisition and extinction in animals and is correlated with response to feared stimuli in both phobic and non-phobic human populations (Flynn et al., 1999). The central amygdala is thought to be more involved in coordination of the *actual* fear response (i.e. autonomic and behavioral responses) whereas the basolateral complex (BLA) is more involved in plasticity related to fear learning (Anglada-Figueroa & Quirk, 2005; Davis, 2006). The BNST is considered part of the "extended amygdala" and is thought to potentially be involved in the long-term experience of fear (Davis & Shi, 1999; Davis, Walker, & Lee, 1997; Davis & Whalen, 2001; Rosen & Donley, 2006; Walker et al.,

2003). The BNST receives input from the medial prefrontal cortex and the central nucleus of the amygdala (Kalin et al., 2005; Straube et al., 2007; Walker et al., 2003). In animal studies, anxiety or fear responses are associated with activation of the BNST (Kalin et al., 2005), while lesioning of the BNST seems to attenuate behavioral signs of anxiety (e.g. Fendt et al., 2003; Waddell, Morris, & Bouton, 2006). Amygdala activation has been reported for phobic symptom provocation in several previous studies (Carlsson et al., 2004; Dilger et al., 2003; Goosens et al., 2007; Schienle et al., 2005). However, there has been only *one* published study reporting BNST activation associated with specific phobia (Straube et al., 2007).

The current study found amygdala activation when comparing spider to butterfly stimuli, in both placebo phobic and placebo control groups. This is consistent with suggestions that amygdala activation is not specific to anxiety disorders or even fear-processing (Stein et al., 2007; Wendt, Lotze, Weike, Hosten, & Hamm, 2007). However, it is important to recognize that in the current study, amygdala activation for the phobic group extended into regions that were not activated in controls. This suggests that the neural response within the amygdala differed between the groups in some way. However, this is most likely not simply a matter of the amygdala being either "on" or "off", but of differences in signaling within subregions of the amygdala. The resolution of fMRI makes it difficult to draw firm conclusions regarding the role of amygdala subregions. However, previous human neuroimaging studies provide evidence that ventral regions of the amygdala are more involved in processing saliency while dorsal regions are more involved in processing fear (Kim, Somerville, Johnstone, Alexander, & Whalen, 2003;

Whalen, Shin, McInerney, Fischer, Wright, & Rauch, 2001). Current results support this theory, revealing the control group to have primarily left *ventral* amygdala activation, while the phobic group exhibited maximum activation in the left *dorsal* amygdala (see Figures 5 and 6). This study is also one of only two studies reporting BNST activation in response to phobic symptom provocation (Straube et al., 2007). This is important in that it corroborates animal research showing the BNST to be important in fear processing. Research in human brain imaging related to anxiety disorders may need to adjust its focus to include not only subregions of the amygdala proper, but also regions within the "extended amygdala", such as the BNST.

The hippocampus is perceived as a central region associated with learning and memory (Neves, Cooke, & Bliss, 2008). It has been shown to be important in both fear conditioning and extinction—especially regarding recall of contextual cues (Corcoran et al., 2005; Maren et al., 1997; Quirk & Mueller, 2008). The hippocampus is thought to modulate amygdala activity through neural connections with both the amygdala and the prefrontal cortex (Corcoran et al., 2005; Maren et al., 1997). Findings from the current study implicate the involvement of the hippocampus in the processing of phobic stimuli and are consistent with findings reported by previous studies (Milad et al., 2007; Paquette et al., 2003; Schienle et al., 2005). It should be noted that many studies have failed to find hippocampal activation (Dilger et al., 2003; Etkin & Wagner, 2007; Straube et al., 2006). However, *both* human and animal studies support the importance of the hippocampus in fear processing. The current findings of hippocampal activation would suggest that the phobic subjects are engaging memory networks. However, this engagement could either

be related to 1) memories of phobic-related cues that were previously learned (and are activated by exposure to *new* phobic stimuli) or 2) the encoding of new memories regarding the presented phobic stimuli and related contextual cues.

Although the importance of the ventral striatum, including the putamen, in reinforcement learning is widely recognized (i.e. Balleine, Delgado, and Hikosaka, 2007; Delgado, 2007; Wickens, Budd, Hyland, & Arbuthnott, 2007), this region has not been a primary focus of research on fear processing (Ollson & Phelps, 2007). There have been consistent findings of putamen, or ventral striatum, activation during symptom provocation in OCD (Kwon et al., 2003; Mataix-Cols et al., 2004; Perani et al, 1995). However, only a few studies have reported putamen activation associated with specific phobia (Schienle et al., 2005). Results reported by recent fear conditioning studies in healthy, non-clinical human populations (using neuroimaging techniques; Jensen et al. 2003; Seymour et al. 2004) and animals (Iordanova et al., 2006) provide reason to believe that the ventral striatum *may* play a role in predictive fear learning and the allocation of attention towards predictors of danger (McNally & Westbrook, 2006). In particular, the putamen may play a role in the coding of stimulus-action (especially motor) associations (Balleine, Delgado, and Hikosaka, 2007). Therefore, putamen activation found during phobic symptom provocation in the current study may relate to 1) the allocation of attention to the spider stimuli as potential predictors of danger or 2) encoding of the association between phobic stimuli and the subjects own responses.

In summary, symptom provocation in spider phobia was associated with unique activations in regions thought to be involved in the direct experience of fear and anxiety

(amygdala and BNST), the prediction of danger (putamen), the cognitive control of emotional responses (PFC), as well as emotional learning and memory (hippocampus, as well as all of the above). These results support the hypotheses stated in specific aim 1.1 and are consistent with findings from previous studies. Therefore, this paradigm should be considered valid for use in examining the effects of DCS on neural activation responses to phobic symptom provocation.

Provocation paradigm: Treatment effects

The primary aim of this study was to investigate the effects of DCS on neural activations during exposure to phobic stimuli. This information is directly relevant to clinical findings that acute DCS administration enhances the effects of exposure (ERP) therapy for anxiety disorders. Results from this study suggest DCS exerts an influence on neural response patterns to phobic stimuli by enhancing prefrontal (PFC) activations for both phobic and non-phobic subjects. This effect seemed more robust when individuals were experiencing a phobic (or emotional) response to the stimuli. DCS did not have an effect on SUDS levels reported during the symptom provocation paradigm. As DCS has not been shown to influence the startle response or initial anxiety/distress to a feared stimulus, this is not surprising (Davis et al., 2006; Ressler et al., 2004; Walker et al., 2002). A recent animal study found that DCS could increase *reconsolidation* of fear memory when exposure to the conditioned stimulus (CS+) was not of long enough duration to achieve extinction (Lee, Milton, & Everitt, 2006). Therefore, it is important that while DCS was not associated with a *decrease* in SUDS through the exposure, it also did not seem to *increase* SUDS levels—and presumably did not further sensitize the

subjects to spiders. DCS was also not associated with any changes in recognition memory for stimuli presented during the symptom provocation paradigm. The null results from behavioral measures suggest that neuroimaging findings are not simply due to changes in behavior or level of anxiety experienced. Therefore, the effects observed are most likely due to the drug itself.

In both phobic and control groups, DCS was associated with increased or unique activations to spiders versus butterflies in the dlPFC and ACC (primarily dorsal in the phobic group; ventral in the control group). In the phobic group, DCS was additionally associated with greater activation in the bilateral inferior frontal gyrus and insula, as well as unique activations in the right OFC. In the control group, there were additional effects of DCS within the left caudate and rostromedial PFC. It should also be noted that, in the DCS-phobic group, right OFC and inferior frontal gyrus activations correlated significantly with severity of phobia (as measured by fMRI SUDS level and BAT, respectively). This is in contrast to the placebo-phobic group, in which primarily amygdala (as well as vlPFC) activations were correlated with symptom severity. Additionally, there was a negative correlation between the left OFC and BLA in the DCS phobic group, whereas a positive correlation between PFC and amygdala activation was found for the placebo phobic group.

As apparent in Figures 5 and 6, the activations for the condition (spider > butterfly) contrast were much more pronounced within both phobic groups than control groups and the treatment interaction effect within the phobic group was also more pronounced than that of the control group. As the primary focus of this study was to

investigate possible mechanisms for DCS-augmented exposure therapy in anxiety disorders, the primary focus of discussion will be on findings from the phobic group. However, it is interesting to note that DCS enhanced primarily dorsal aspects of the ACC in phobics, but enhanced primarily ventral ACC in controls. The dorsal aspects of the ACC are considered to be involved in more "cognitive" tasks whereas the ventral ACC is involved in tasks that are more emotional in nature (Bush, Luu, and Posner, 2000). It is possible that the regional influence of DCS within the ACC depends upon the emotional arousal of subjects. If the task produces high emotional arousal, DCS may serve to enhance cognitive processing of stimuli through enhanced dorsal ACC activation. If the task is salient, but not necessarily emotional arousing, DCS may serve to enhance emotional processing of the stimuli through enhanced ventral ACC activation. The differential effects of DCS on behavioral, cognitive, and biological functioning in various clinical and non-clinical populations, and during tasks of varying emotional intensity should be examined by future research.

The effects of DCS within the phobic group were driven both by increased activations to spider stimuli and decreased activations to butterfly stimuli (compared to the placebo-treated phobics). DCS, therefore, seems to differentially influence the processing of both feared and non-feared stimuli, presented within the same session. This may relate to an increase in selective attention for salient and fear-provoking stimuli—decreasing distractions from the fear extinction process. Alternatively, this could relate to a decrease in anticipatory anxiety between exposure blocks. Either way, it suggests that

DCS does not serve to simply enhance brain activation to *all* stimuli during the time in which the drug is active within the central nervous system (CNS).

Current findings suggesting DCS can enhance PFC activation is consistent with recent animal research suggesting DCS mediates NMDA-receptor activity within the medial PFC (mPFC; Fujihira et al., 2007; Millecamps et al., 2007; Murai et al., 2007). However, in animal research examining DCS effects on fear extinction, the prefrontal cortex has taken a back seat to the amygdala and hippocampus. The only other human neuroimaging study to investigate the effects of acute DCS did not examine changes in regions of the PFC—only the amygdala (Britton et al., 2007). Therefore, the current study provides initial, preliminary evidence that activity in regions within the PFC are enhanced by DCS administration during fear processing. Future animal and human research of DCS-augmented fear extinction should further evaluate effects within various regions of the PFC.

Animal research on fear learning in general (not involving DCS) suggests the PFC is required for fear extinction and memory for extinction (Anglada-Figueroa & Quirk, 2005; Morgan, Schulkin, & LeDoux, 2003). As mentioned previously, most previous research has focused on the inhibitory influences of the *medial* PFC in fear processing, though there have been studies suggesting the *lateral* PFC plays a similar role (Dunsmoor et al., 2007; Lacroix et al., 2000; Milad et al, 2006; Milad & Quirk, 2002; Schienle et al., 2005; Paquette et al., 2003). The current study found DCS to be associated with enhanced activations in primarily *lateral* regions of the PFC and OFC, as well as areas of the dorsal ACC. Increased lateral PFC activation has been associated with reappraisal, distraction,

and other cognitive strategies to regulate emotional response (Kalisch et al., 2005, 2006). It is possible that DCS is increasing the use of such strategies—or at least the ability of individuals to engage in such strategies if directed to do so.

The OFC has extensive connections with the dlPFC as well as the amygdala and is considered important in stimulus-reinforcement association learning—altering behavior in response to feedback (Kringelbach & Rolls, 2004; Pears, Parkinson, Hopewell, Everitt, and Roberts, 2003; Stein et al., 2007). The lateral OFC in particular is thought to be involved in mediating responses during *negative* affective states (Milad & Rauch, 2007). The dorsal ACC is thought to be involved in cognitive processing as well and is important in the generation of error signals and allocation of attentional resources (Critchley, 2005; Phillips et al., 2003). Therefore, enhancement of PFC, OFC, and dorsal ACC activations by DCS may increase rational, cognitive processing and the ability to modulate responses to negative emotional stimuli. The negative correlation between OFC and amygdala activation within the DCS phobic group, provides further evidence that DCS may involve enhanced *modulation* by prefrontal regions. Enhanced PFC activation may serve as a common mechanism for recent findings showing DCS-augmented ERP to beneficially influence both anxiety and depressive symptoms (Wilhelm et al., 2008).

In recent years, the insula has received increasing focus within the anxiety disorder literature (Paulus & Stein, 2006; Simmons, Matthews, Paulus, and Stein, 2008; Stein et al., 2007). The insula has been consistently reported to activate during phobic symptom provocation paradigms (Dilger et al., 2007; Schienle et al. 2007; Straube et al., 2007) and this activity has been shown to normalize with successful treatment (Goosens

et al., 2007; Straube et al, 2006). The insula is understood to be involved in interoceptive function and the integration of internal body states with external events (Cechetto, 1994; Craig, 2002; Phillips et al., 2003). Paulus and Stein (2006) recently presented an insula hypothesis of anxiety disorders, implicating the insula as a primary locus of dysfunction. They postulate that individuals with anxiety disorders have altered interoception that includes an altered prediction signal regarding expected body state (i.e. in response to certain objects or situations). The neural system that underlies interoception converges in the anterior insula (Craig, 2002). Additionally, the insula lies between and has strong connections with areas of the PFC (including the ACC) and the amygdala (Craig, 2002). Therefore, it is optimally located to receive signals concerning saliency of environmental stimuli and how such stimuli may affect bodily states. Information is relayed from the insula to the ACC, which is involved in the generation of error signals and allocation of attentional resources. The dorsal aspects of the ACC are thought to particularly be involved in the integration of cognitive processes with autonomic arousal (Critchley, 2005; Phillips et al., 2003).

Increased activation in both the insula and dorsal ACC with DCS may reflect a more complete integration of information regarding various aspects of the fear experience. This could relate to a stronger cognitive representation of the fear itself (as discussed further in the next paragraph)—enabling the individual to integrate their bodily state predictions with the actual response experienced. Such an effect could presumably have a strong influence on the learning process during extinction, strengthening new associations between stimulus and response. Originally, it was hypothesized that insula,

along with amygdala, activation would decrease with the administration of DCS. This was based on knowledge that the insula and amygdala share extensive connections with one another, and the idea that both structures are involved in the initial response to emotionally-salient stimuli (Phillips et al, 2003). The current finding that, during initial symptom provocation, acute DCS significantly *enhanced* insula, but not amygdala, activation suggests that each of these regions play a distinct role in fear processing.

It has been shown that DCS increases generalization of fear extinction (i.e. after fear extinction to one CS+, fear response to a second CS+ is also decreased; Ledgerwood, Richardson, & Cranney, 2005). Researchers have postulated that this effect may be due to a devaluing of the UCS itself. In other words, the DCS-treated mice become extinguished to not simply the CS+, but also to their *cognitive representation* of the UCS (Davis et al., 2006; Ledgerwood et al., 2005). In fact, animal studies have shown the generalization effects observed for DCS are very similar to the effects observed after extinction sessions with the UCS (Ledgerwood et al., 2005). It is possible that this is the effect we are seeing in the current study and that the increased PFC, ACC, and insula activations represent higher level processing in which abstract cognitive representations of spiders (and the fear of spiders) is held in mind. This would be in contrast to the processing of only concrete aspects of the picture stimuli.

In the human anxiety disorder literature, it has been postulated that there are two required elements of successful ERP therapy: 1) the fear structure must be activated and 2) information incompatible with the fear prediction must be incorporated (Foa & Kozak, 1989). The "fear structure" is thought to involve information related to the feared

situation, information about predicted and actual responses to such stimuli (i.e. verbal, physiological, and behavioral responses), and *interpretations* regarding the meaning of the stimulus-response elements (Foa & Kozak, 1989; Lang, 1977, 1979; Rodebaugh and Chambless, 2004;). Therefore, exposure therapy is thought to rely on activation of a cognitive representation of the core fear—not simply activation of sensory and attentional resources to the stimulus presented. For example, ERP for a spider phobic patient may involve exposure to words associated with spiders (i.e. "spider", "creepy", "bites"), pictures and videos of spiders, and live spiders. These individual exposure stimuli are all used to elicit the core fear of spiders and spider bites. During every exposure, the patient is encouraged to focus on thoughts related to that core fear and related stimulus-response predictions (i.e. "Spiders are gross and disgusting. They are dangerous and will bite me. The bite will be painful and it will kill me."). The power of ERP most likely comes from habituation to that core fear and related catastrophic stimulus-response interpretations—not simply to the individual, concrete stimuli.

DCS appears to enhance activation of PFC, ACC, and insula regions, potentially allowing patients to focus more easily on the cognitive representations of the core fear—including information about predicted and actual responses to such stimuli, and *interpretations* regarding the meaning of the stimulus-response elements. This hypothesis is consistent with the fMRI data, but should be considered preliminary because there were no behavioral measures in the current study to support or refute it. Such a difference in cognitive processing would not necessarily cause changes in initial anxiety level or alter memory for concrete aspects of the exposure (i.e. the spider pictures). Kamphuis and

Telch (2000) reported that greater focus on core threats (as well as less distraction) during exposure was associated with greater fear reduction. Additional studies have reported memory for anxious responses and abstract information during exposure relates to treatment outcome (Kamphuis and Telch, 2000; Kindt et al., 1999; Zoellner, Echiverri, and Craske, 2000). Future studies investigating DCS-augmented ERP therapy should include measures related to higher-level, more abstract cognitive processing such as those used in the studies mentioned.

Animal studies examining DCS effects on fear learning have primarily utilized either systemic administration or intracerebral infusion directly into the basolateral amygdala (Davis et al., 2006; Ledgerwood, Richardson, & Cranney, 2003, 2004; Walker et al., 2002). There is evidence that NMDA receptor activity within both the hippocampus and amygdala is influenced by DCS (Rouaud and Billard, 2003; Yaka et al., 2007; Yang & Lu, 2005; Yamamoto et al., in press). Current theories of DCS revolve around enhancement of the *consolidation* of memory extinction—a process known to involve the hippocampus and amygdala (Corcoran et al., 2005; Davis et al., 2006; Hofmann et al., 2006b; Hofmann, 2007; Maren et al., 1997; Quirk & Mueller, 2008; Richardson et al., 2004). The only other neuroimaging study conducted to examine the effects of acute DCS administration, reported DCS to decrease overall amygdala activation (Britton et al., 2007). Although in the current study, amygdala and hippocampal activations met criteria for significance *only* in the placebo groups (and not the DCS groups), the effect of treatment was *not* statistically significant in these regions.

The contradictory findings between the current study and those of Britton et al. (2007) may be due to differences in the study population and/or the dosage of DCS. The Britton et al. study included only non-anxious healthy control subjects whereas the current study included an anxiety-disordered population. Current results suggest that DCS may modulate activity differently in phobic and non-phobic populations—enhancing more dorsal PFC regions in phobic and more ventral regions in controls. The current study administered 100 mg DCS whereas Britton et al. used 500 mg. There is reason to believe, from both animal and human literature, that higher doses of DCS are less effective for enhancing fear extinction (Rothbaum, 2008). Therefore, different doses of DCS may have different effects on neural and cognitive processes.

Differences in results between animal and human neuroimaging studies of acute DCS administration may be due to differences in non-primate and human brain anatomy and function. It is possible that DCS influences the neural network in humans differently than in animals—causing the PFC, rather than the hippocampus and amygdala, to assume the role of creating and consolidating new association memories. It is also possible that contradictory results are due to the fact that most animal studies examine the entire fear extinction process whereas the current study only examined the initial phase of fear extinction, symptom provocation. It is possible that DCS is associated with modulation of hippocampal and amygdala activation in humans during later phases of the fear extinction process.

In summary, the current findings suggest that DCS causes an increase in prefrontal (lateral PFC and OFC, dorsal ACC) and insula activation—possibly enhancing

the cognitive processing of feared stimuli (including interoceptive and attentional awareness), the modulation of responses to those stimuli (autonomic, cognitive, and/or behavioral), and the learning of stimulus-response associations.

Study Limitations

The current study did not include a CBT treatment arm, nor did it include complete in-session habituation to phobic stimuli. It is therefore possible that the neural effects reported for DCS in the current study are not the primary mechanisms through which DCS enhances fear extinction. DCS may exert a different influence during various phases of fear extinction (including acquisition, habituation, and maintenance). Future research could address this possibility by examining the effects of DCS during *repeated* sessions of symptom provocation, conducted pre- and post- ERP treatment. Additionally, the effects of DCS could be examined using an fMRI habituation paradigm involving more prolonged exposure to stimuli. This would allow examination of DCS effects during multiple phases of fear extinction.

The current sample size (though comparable to those of other fMRI studies) was most likely not sufficient to provide complete characterization of behavioral and neuropsychological differences between groups. Therefore, results related to the effects of phobia diagnosis and DCS administration on cognitive functioning require replication for any specific conclusions to be made.

Conclusions

The current study represents the first investigation of acute DCS effects on neural processing during phobic symptom provocation. It is also the first study to examine acute

DCS effects on neuropsychological functioning. Results are important in providing direction for future research examining the use of acute DCS administration in enhancing fear extinction, exposure therapy, and cognitive functioning in general.

Results suggest that specific phobia may be associated with subtle differences in cognitive functioning. The current study also provides evidence that DCS may have subtle effects on emotional decision-making. Further research is needed to examine cognitive functioning in specific phobia and the potential neuropsychological effects of DCS.

The primary finding of the current study was that DCS enhances PFC, cingulate, and insula activations during phobic symptom provocation. Enhancement of activation in these regions may relate to higher-level, cognitive processing and the integration of interoceptive information during emotional processing. Future animal and human research with DCS should further investigate how DCS may modulate activity in these regions, as well as how these activations relate to changes in emotion, cognition, and behavior.

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Table 1.

Abbreviations

Region	Abbreviation
Neuroanatomical	
Region of interest	ROI
Prefrontal cortex	PFC
medial	mPFC
dorsolateral	dlPFC
ventrolateral	vlPFC
Orbitofrontal cortex	OFC
Anterior cingulate cortex	ACC
Parahippocampal gyrus	PHG
Bed nucleus of the stria terminalis	BNST
Brodmann area	BA
Measures	
Subjective Units of Distress	SUDS
Behavioral Avoidance Test	BAT
Spider Phobia Questionnaire	SPQ
Magnetic resonance imaging	MRI
Functional MRI	fMRI
Blood oxygenation level dependent	BOLD
Positron emission tomography	PET
Other	
D-cycloserine	DCS
Exposure and Response Prevention	ERP
Conditioned stimulus	CS
Unconditioned stimulus	UCS

Table 2.

Demographic and Clinical Measures

		Non-phobic control groups		Spider phobic groups		Significance	
		1. Placebo (N=11)	2. DCS (N=12)	3. Placebo (N=13)	4. DCS (N=13)	Contrast	p
Demographic							
Age	M (SD)	23.09 (3.70)	26.75 (9.09)	25.85 (7.73)	24.23 (6.22)		
Education	M (SD)	14.91 (1.76)	15.75 (1.87)	15.23 (2.17)	14.92 (1.98)		
Sex	M/F	3/8	4/8	6/7	1/12		
WAIS-Vocab	M (SD)	51.36 (8.15)	51.92 (6.43)	53.08 (5.77)	49.16 (6.74)		
WAIS-MR	M (SD)	21.27 (3.04)	21.50 (3.42)	22.31 (2.25)	21.85 (2.91)		
Clinical							
SPQ raw	M (SD)	1.09 (.94)	1.00 (1.21)	20.15 (3.21)	22.38 (3.20)	(3&4) > (1&2)	.000**
BAT score	M (SD)	11.82 (.60)	1.50 (.80)	2.31 (1.38)	1.92 (1.38)	(1&2) > (3&4)	.000**
BAT SUDS	M (SD)	.91 (.86)	.71 (.86)	4.38 (1.21)	4.85 (1.09)	(3&4) > (1&2)	.000**
BDI	M (SD)	1.67 (2.65)	.50 (.71)	2.54 (3.15)	1.77 (1.92)		

Abbreviations: Education = Years of Education; WAIS = Wechsler Adult Intelligence Scale; Vocab = WAIS vocabulary subtest; MR = WAIS matrix reasoning subtest; SPQ = Spider Phobia Questionnaire; BAT = Behavioral Avoidance Test; SUDS = Subjective Units of Distress (during the BAT); BDI = Beck Depression Inventory; ** = significant at p<.01, * = significant at p<.05.
1 = placebo-control group, 2 = DCS-control group, 3 = placebo-phobic group, 4 = DCS-phobic group.

Table 3.

Adverse Symptom Checklist

		Non-phobic control groups		Spider phobic groups		Significance	
		1. Placebo (N=11)	2. DCS (N=12)	3. Placebo (N=12)	4. DCS (N=13)	Contrast	p
ASC score	M (SD)	.88 (1.36)	.25 (.46)	2.40 (2.99)	4.10 (4.43)	(3&4) > (1&2)	.009
Opinion	Placebo/DCS	9/2	12/0	7/5	7/6	(1&2) > (3&4)	.009
						2 > 4	.015
Accuracy	Correct/Incorrect	9/2	0/12	7/5	6/7	(1&3) > (2&4)	.003
						4 > 2	.015

Abbreviations: ASC= Adverse Symptom Checklist; ** = significant at p<.01, * = significant at p<.05.

Table 4.

Neuropsychological Data: Rey Osterrieth Complex Figure Test

		Non-phobic control groups		Spider phobic groups		Significance	
		1. Placebo (N=11)	2. DCS (N=12)	3. Placebo (N=13)	4. DCS (N=13)	Contrast	p
Accuracy							
Copy	M(SD)	35.36 (.92)	34.42 (3.68)	34.46 (2.14)	34.62 (1.98)		
Immediate	M(SD)	26.45 (4.34)	25.79 (5.80)	26.17 (3.76)	26.08 (5.67)		
Delayed	M(SD)	26.23 (4.12)	24.29 (6.52)	25.35 (3.73)	25.85 (5.52)		
Organization							
Copy	M(SD)	5.18 (1.17)	5.17 (1.34)	3.69 (1.97)	4.23 (2.01)	(1&2)>(3&4)	.024*
Immediate	M(SD)	5.64 (1.21)	5.00 (1.04)	4.58 (1.44)	4.62 (1.66)		
Delayed	M(SD)	5.64 (.92)	5.00 (1.21)	4.62 (1.26)	4.38 (1.98)		

Abbreviations: RCFT = Rey-Osterrieth Complex Figure Test; Acc = Accuracy; Immed = Immediate recall; Delay= delayed recall; ** = significant at p<.01, * = significant at p<.05.

Table 5.

Neuropsychological Data: Iowa Gambling Test

		Non-phobic control groups		Spider phobic groups		Significance	
		1. Placebo (N=11)	2. DCS (N=12)	3. Placebo (N=12)	4. DCS (N=13)	Contrast	p
IGT Total	M(SD)	53.27 (9.89)	34.00 (27.46)	28.58 (21.88)	21.85 (28.42)	(1&2)>(3&4)	.009*
Block 1	M(SD)	.73 (10.25)	-5.50 (9.35)	-1.83 (10.14)	-2.31 (7.25)		
Block 2	M(SD)	9.36 (9.28)	4.25 (7.61)	3.58 (9.26)	8.17 (8.16)		
Block 3	M(SD)	11.27 (7.96)	12.17 (6.69)	8.17 (8.16)	4.31 (10.61)	(1&2)>(3&4)	.032*
Block 4	M(SD)	16.00 (4.00)	12.67 (8.96)	9.50 (5.73)	6.92 (11.76)	(1&2)>(3&4)	.015*
Block 5	M(SD)	16.55 (5.52)	11.50 (9.91)	7.00 (10.63)	6.46 (6.94)	(1&2)>(3&4)	.005*

Abbreviations: IGT = Iowa Gambling Test; ** = significant at p<.01, * = significant at p<.05.

Table 6.

Neuropsychological Data: Wisconsin Card Sorting Test

		Non-phobic control groups		Spider phobic groups	
		1. Placebo (N=11)	2. DCS (N=12)	3. Placebo (N=13)	4. DCS (N=12)
WCST Total	M(SD)	65.73 (2.87)	69.17 (8.11)	69.23 (8.96)	70.00 (6.61)
Errors	M(SD)	11.18 (3.43)	12.67 (7.15)	18.23 (16.58)	15.67 (9.33)
Perseverative	M(SD)	6.82 (1.99)	7.58 (4.36)	10.54 (10.14)	9.08 (5.43)
Sets Completed	M(SD)	6.00 (.00)	6.00 (.00)	5.46 (1.39)	6.00 (.00)
Sets Lost	M(SD)	.27 (.47)	.50 (.80)	.54 (.97)	1.25 (2.90)

Abbreviations: WCST = Wisconsin Card Sorting Test; Sets Comp = Sets completed; Persev = Perseverative errors.

Table 7.

Neuropsychological Data: Wechsler Memory Scale III

		Non-phobic control groups		Spider phobic groups	
		1. Placebo (N=11)	2. DCS (N=12)	3. Placebo (N=13)	4. DCS (N=12)
Logical Memory					
I recall	M(SD)	52.73 (8.34)	49.25 (9.60)	52.69 (10.30)	54.38 (5.45)
II recall	M(SD)	34.45 (6.31)	32.67 (7.30)	34.85 (8.21)	36.38 (3.93)
II retention	M(SD)	88.35 (9.88)	94.28 (4.82)	92.75 (9.64)	94.09 (12.33)
Faces					
I recall	M(SD)	39.55 (2.98)	41.83 (2.86)	41.00 (4.28)	42.23 (3.94)
II recall	M(SD)	42.00 (2.28)	41.67 (3.11)	41.62 (3.43)	42.00 (4.12)
II retention	M(SD)	106.67 (8.58)	99.83 (7.84)	102.30 (11.56)	99.66 (8.23)

Abbreviations: WMS = Wechsler Memory Scale; LM I = Logical Memory I; LM II = Logical Memory II; LM Ret = Logical Memory retention between I and II; Faces Ret = Retention for faces memory between I and II.

Table 8.

Behavioral data from fMRI provocation paradigm

		Non-phobic control groups		Spider phobic groups		Significance	
		1. Placebo (N=11)	2. DCS (N=12)	3. Placebo (N=13)	4. DCS (N=12)	Contrast	p
SUDS							
Pre-Scan	M(SD)	.00 (.00)	.00 (.00)	2.88 (1.70)	3.36 (1.86)	(3&4)>(1&2)	.000**
Scan 1	M(SD)	.14 (.32)	.08 (.29)	5.42 (1.10)	6.09 (1.16)	(3&4)>(1&2)	.000**
Scan 2	M(SD)	.00 (.00)	.08 (.29)	5.54 (1.41)	6.09 (.58)	(3&4)>(1&2)	.000**
Recognition Memory							
Spiders D'	M(SD)	53.09 (16.60)	54.00(13.27)	65.33 (11.86)	62.91 (13.98)	(3&4)>(1&2)	.014*
Butterflies D'	M(SD)	59.27 (6.89)	61.67 (7.33)	61.00 (8.88)	61.09 (11.333)		
Total D'	M(SD)	56.18 (9.31)	57.00 (6.06)	63.17 (7.31)	62.00 (10.08)	(3&4)>(1&2)	.018*

Abbreviations: SUDS-Subjecive Units of Distress on a scale of 0 – 8; ** = significant at p<.01, * = significant at p<.05.

Table 9.

Placebo phobic > placebo control (RFX, p<.001)

Side	Region	# voxels	Max T	x	y	z
Spiders > Butterflies, RFX .001						
left	dlPFC (BA 9)	15	-4.54	-48	23	31
right	frontal operculum	8	-4.21	48	-16	19
left	frontal operculum	10	-4.41	-45	-13	16
right	insula	9	-4.70	33	-16	13
left	putamen	4	4.56	-24	-7	1
right	parahippocampal gyrus	16	-4.80	24	-25	-20
right	bed nucleus of stria terminalis	4	4.42	9	-1	-2
Spiders > low-level baseline						
right	frontal operculum	72	5.26	45	14	-2
right	frontal operculum	14	4.70	45	-1	1
right	frontal operculum	30	4.31	36	20	1
left	frontal operculum	3	4.21	-42	11	7
mid	dorsal cingulate	15	4.24	0	5	40
left	insula	15	4.72	-27	17	-5
left	insula	58	4.39	-30	17	10
right	insula	114	6.03	39	-10	-8
left	medial caudate	13	4.48	-15	5	1
right	medial caudate	3	4.07	12	11	10
left	putamen	7	4.13	-24	-4	1
right	bed nucleus of stria terminalis	15	4.88	9	-1	-2
Butterflies > low-level baseline						
right	frontal operculum	29	4.58	42	-4	4
left	frontal operculum	11	4.24	-33	14	16
left	posterior cingulate	33	4.64	-3	-31	34
left	anterior cingulate	43	4.67	-6	29	10
right	posterior cingulate	26	5.24	12	-16	37
left	isthmus of cingulate gyrus	33	5.28	-9	-49	4
left	insula	54	5.32	-36	-25	10
right	insula	26	5.32	42	-13	7
right	insula	7	3.98	39	-7	10
left	insula	15	4.38	-39	-4	10
left	medial caudate	7	4.35	-24	8	7
right	putamen	17	4.75	15	14	1

Table 10.

DCS phobic > Placebo phobic (RFX, p<.001)

Side	Region	# voxels	Max T	x	y	z
Spiders > Butterflies						
left	dlPFC (BA 9)	5	4.41	-36	29	31
left	dlPFC (BA 46)	4	4.00	-48	23	32
left	inferior frontal (BA 44)	41	4.47	-48	23	26
right	inferior frontal (BA 45)	16	4.67	48	8	28
right	inferior frontal (BA 45)	9	4.24	36	-13	46
right	cingulate (BA 24)	6	3.98	12	14	34
right	insula	4	3.86	33	-16	13
left	insula	47	6.19	-39	-7	10
left	insula	4	4.15	-39	-16	-5
Spiders > low-level baseline						
left	inferior frontal (BA 45)	22	4.88	-48	23	22
right	basal operculum	3	3.98	30	20	-8
right	dlPFC (BA 9)	4	4.14	27	41	31
left	presubiculum	23	-4.66	-18	-22	-14
Butterflies > low-level baseline; RFX .001						
left	inferior frontal (BA 44)	96	-4.75	-33	1	31
left	dlPFC (BA 9)	4	-3.94	-18	35	31
left	anterior cingulate	48	-5.61	-3	-31	34
right	isthmus of cingulate gyrus	3	-4.13	6	-46	10
right	subiculum	19	-4.34	24	-37	-5
left	parasubiculum	2	-4.00	-18	-13	-20
right	claustrum	9	-4.21	30	-22	4
left	medial caudate	7	-4.64	-24	8	7

Table 11.

Spiders > Butterflies: main effect for each group separately (RFX, p < .001)

Side	Region	# voxels	Max T	x	y	z
Placebo Phobic						
right	vlPFC (BA 45)	91	8.22	45	32	10
right	dlPFC (BA9)	28	7.35	42	5	37
left	frontal operculum	4	-5.49	-48	-13	16
left	frontal operculum	7	5.23	-45	23	7
right	frontal operculum	15	5.47	45	2	1
right	insula	6	5.41	36	2	1
right	posterior insula	61	-9.59	33	-16	13
right	compact insular claustrum	5	5.71	30	11	1
left	limitans claustrum	17	6.88	-33	-13	-5
left	lateral amygdala	531	9.06	-24	-4	-14
(cluster also includes central and basomedial amygdala)						
right	basomedial amygdala	45	5.97	18	-1	-11
right	central amygdala	4	4.72	24	-4	-11
right	bed nucleus of the stria terminalis	5	4.93	12	-1	-5
right	CA1 field of the hippocampus	35	6.37	27	-10	-14
left	CA3 field of hippocampus	10	5.56	-24	-19	-14
left	ventral putamen	8	5.33	-30	-22	-5
Placebo Control						
right	inferior frontal gyrus (BA 45)	7	5.59	45	-1	31
left	OFC	6	5.37	-30	32	-5
left	superior frontopolar (BA 10)	6	-5.40	-18	59	1
left	frontal operculum	5	5.33	-45	2	1
right	insula	3	5.43	39	-7	1
right	insula	11	5.12	36	2	1
right	preamygdalar claustrum	16	5.32	27	-1	-11
left	lateral amygdala	20	5.29	-27	-4	-20
DCS Phobic						
left	inferior frontal (BA 45)	9	5.21	-45	20	22
right	inferior frontal (BA 44)	5	4.89	60	11	16
right	inferior frontal (BA 45)	35	5.79	54	5	25
right	inferior frontal (BA 44)	12	5.17	45	8	28
left	dlPFC (BA 9)	3	5.32	-33	35	34
right	dlPFC (BA 9)	41	5.68	36	29	31
left	dlPFC (BA 9)	760	8.00	-24	44	28
right	dlPFC (BA 46)	5	5.28	39	41	19
right	dlPFC (BA 46)	8	5.60	45	35	13
right	dlPFC (BA 46)	19	5.47	42	41	14
right	inferior OFC	9	5.21	45	17	-5
left	lateral OFC	14	5.00	-33	35	-11
right	frontal operculum	98	5.87	39	26	1
right	frontal operculum	167	5.71	46	5	4
right	frontal operculum	3	5.27	39	-1	10
right	frontal operculum	313	8.22	36	17	13
right	frontal operculum	3	4.72	48	14	-2
left	frontal operculum	20	5.47	-45	2	1

Table 11.

Spiders > Butterflies: main effect for each group separately (RFX, p < .001), Continued

Side	Region	# voxels	Max T	x	y	z
DCS Phobic						
right	dorsal cingulate (BA 32)	6	5.16	6	17	31
left	dorsal cingulate (BA 32)	213	6.78	-3	17	31
right	dorsal cingulate (BA 24)	2922	8.79	6	2	46
left	insula	299	8.03	-30	20	10
right	insula	124	6.68	36	2	4
right	caudate	637	9.67	15	-10	13
left	dorsal claustrum	7	4.93	-33	-13	-2
right	putamen	50	5.75	27	-19	1
right	medial putamen	46	6.54	24	5	7
right	parahippocampal gyrus	52	6.20	24	-43	-8
DCS Controls						
L/mid	superior rostral gyrus (BA 10)	298	6.39	-65	6	4
left	vlPFC (BA 45)	9	5.60	-48	26	7
left	dlPFC (BA 9)	25	5.23	-42	5	43
left	dmPFC (BA 9)	5	5.20	-6	50	34
left	lateral OFC	21	5.12	-36	29	-11
left	frontal operculum	30	5.64	-39	20	10
left	posterior cingulate (BA 23)	7	4.80	-3	-22	34
left	anterior cingulate (BA 32)	40	6.90	-15	44	10
left	parahippocampal gyrus	17	5.23	-24	-34	-14
left	CA1 field hippocampus	7	-4.85	-30	-40	-2
right	piriform cortex	6	5.33	27	11	-11

Figure Captions

Figure 1. Theoretical neural circuitry model of anxiety

Figure 2. Average Iowa Gambling Test (IGT) block scores for all four groups.

Figure 3. BOLD response for selected regions found to have greater activation for the spider than the butterfly condition in the placebo phobic group but not the placebo control group.

Figure 4. BOLD response for selected regions found to be differentially activated for the placebo-treated and DCS-treated phobic groups when comparing the spider and butterfly conditions.

Figure 5. Regional activations for the spiders > butterflies contrast in a) placebo-treated phobic group, b) DCS-treated phobic subjects, and c) treatment interaction.

Figure 6. Regional activations for the spiders > butterflies contrast in a) placebo-treated control group, b) DCS-treated control group, and c) treatment interaction.

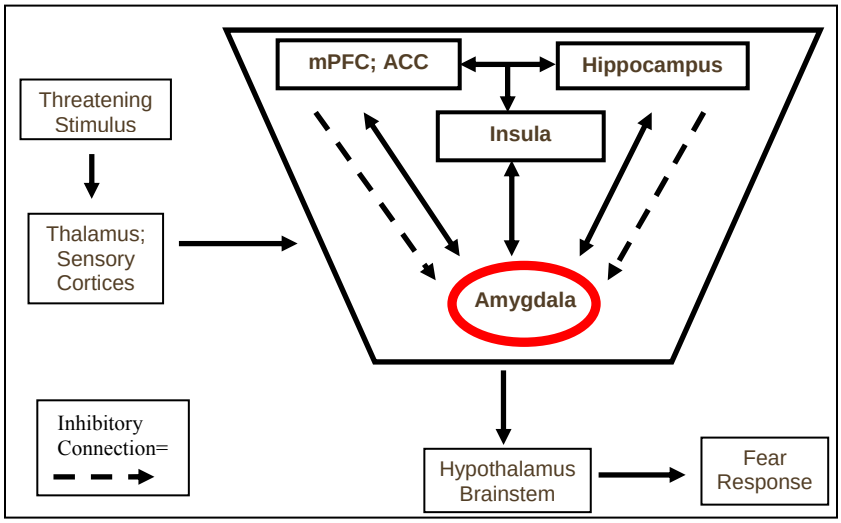


Figure 1. Theoretical neural circuitry model of anxiety

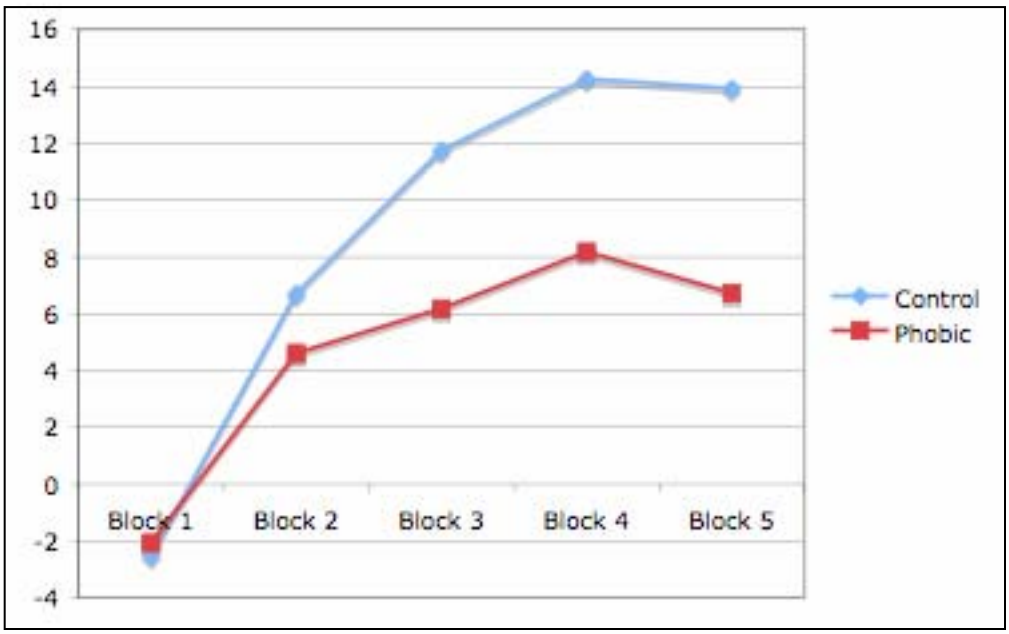


Figure 2. Average Iowa Gambling Test (IGT) block scores for phobic and control groups. The phobic group as a whole performed worse than the control group in blocks 3 ($t(46)=2.28$, $p=.027$), 4 ($t(46)=2.54$, $p=.014$), and 5 ($t(46)=2.92$, $p=.005$).

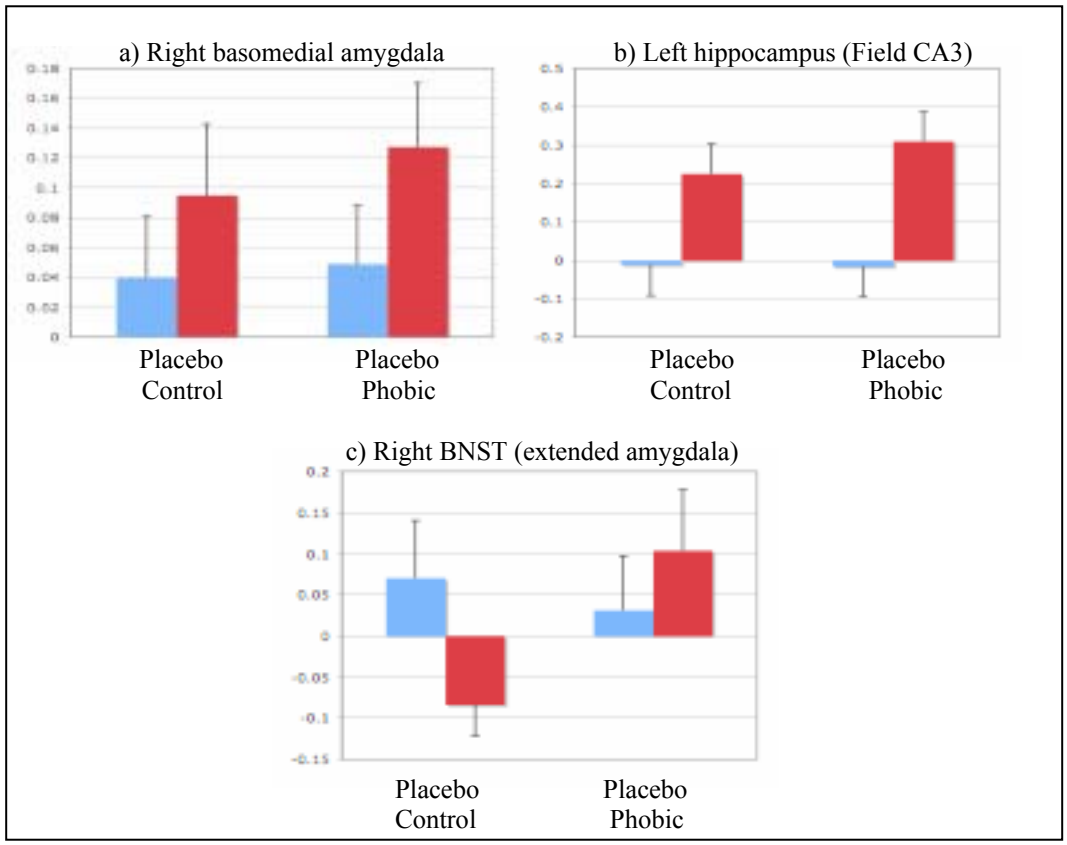


Figure 3. BOLD response (represented by & signal change) for selected regions found to have greater activation for the spider than the butterfly condition in the placebo phobic group but not the placebo control group: a) right basomedial amygdala (t(11)=5.97, x,y,z=18,-1,-11), and b) left hippocampus (t(11)=5.56, x,y,z=-24,-19,-14). BOLD response for a region of the extended amygdala is also shown, which was significant for the condition (spider > butterfly) x diagnosis (phobic > control) interaction: c) BNST (t(21)=4.42, x,y,z=9,-1,-2).

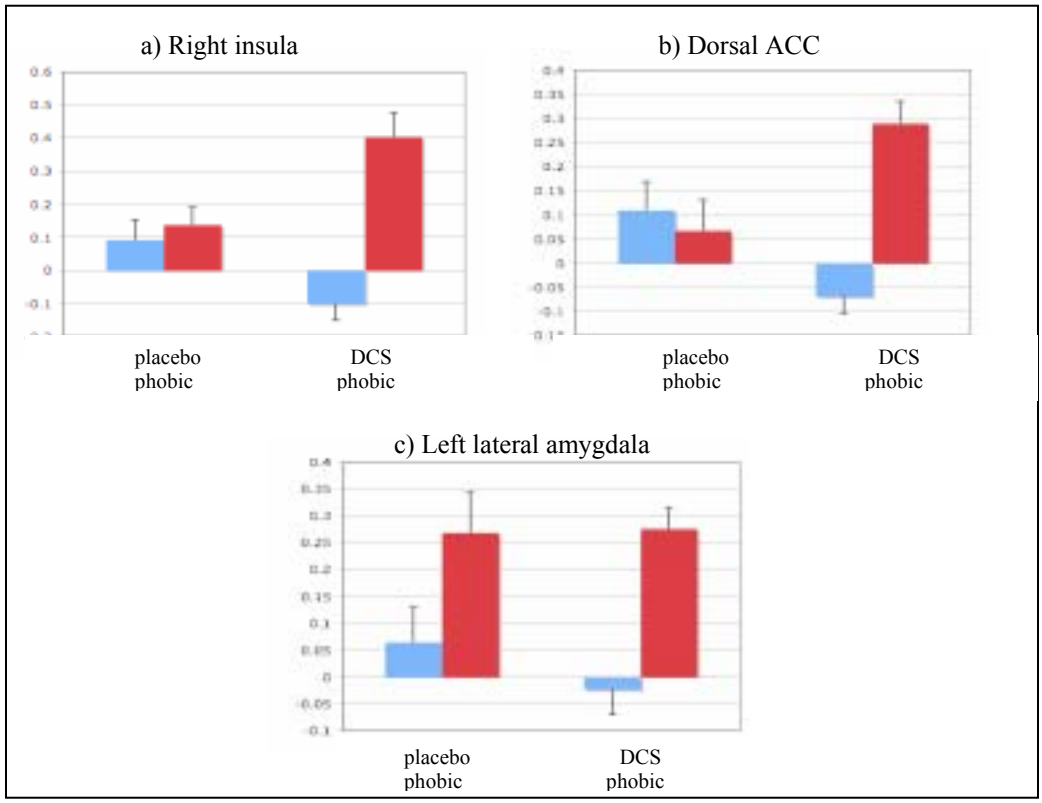
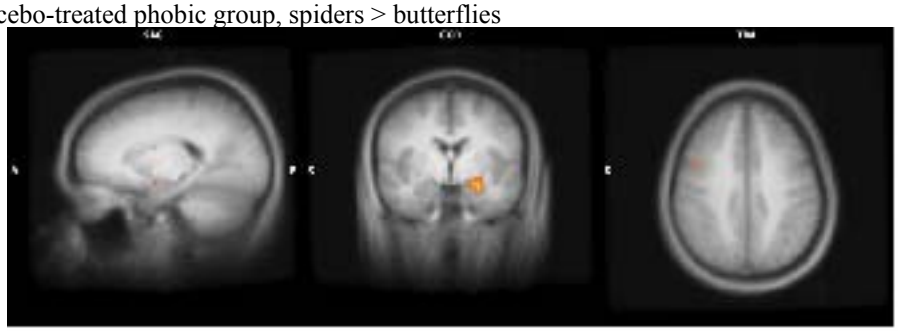
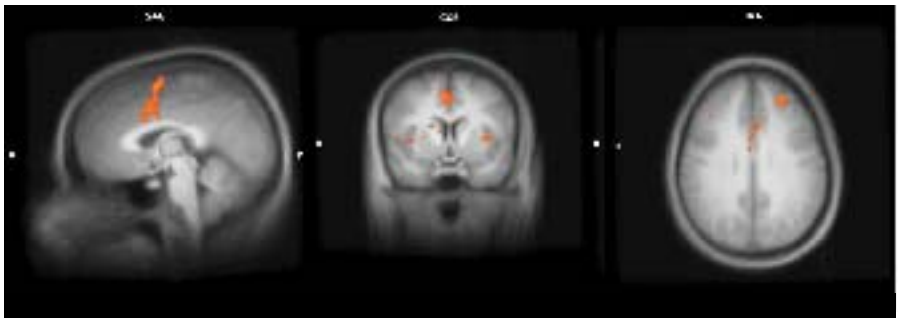


Figure 4. BOLD response (represented by & signal change) for selected regions found to be differentially activated for the placebo-treated and DCS-treated phobic groups when comparing the spider and butterfly conditions: a) right insula (t(21)=3.86, x,y,z=33,-16,13), and b) dorsal ACC (t(21)=3.98, x,y,z=12,14,34). BOLD response is also shown for a selected region of the amygdala, which met criteria for significance in the placebo-treated but not the DCS-treated phobic group: c) left lateral amygdala (t(11)=9.06, x,y,z=-24,-4,-14).

a) Placebo-treated phobic group, spiders > butterflies



b) DCS-treated phobic group, spiders > butterflies



c) Treatment group (DCS > placebo) X Condition (spiders > butterflies) interaction

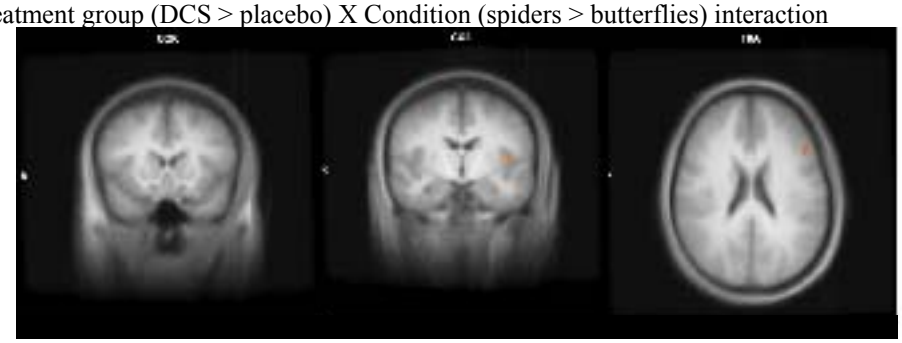
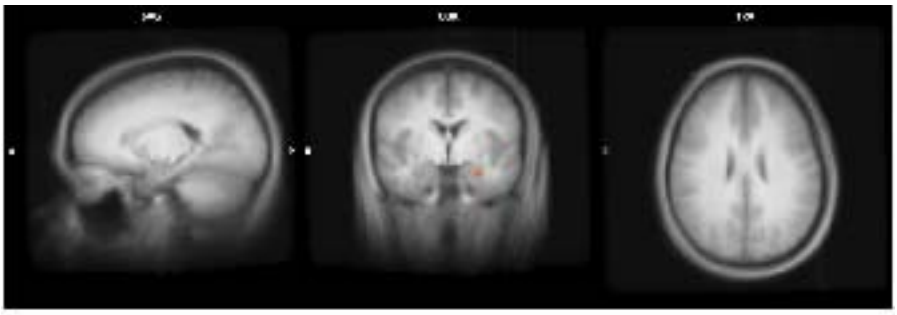
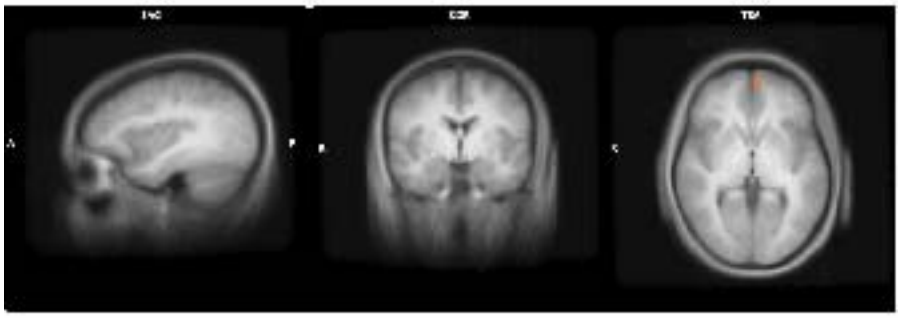


Figure 5. Regional activations for the spiders > butterflies contrast in: a) placebo-treated phobic group; bilateral lateral amygdala (sagittal and coronal images; t=9.06, x,y,z=-24,-4,-14), right dlPFC (axial image, BA 9, t(11)=7.35, x,y,z=42,5,37); b) DCS-treated phobic subjects; dorsal cingulate (all 3 images; BA 32, t(10)=6.78, x,y,z=-3,17,31), bilateral dlPFC (transverse/axial image; BA 9, t(10)=8.00, x,y,z=-24,44,28), bilateral insula (t(10)=8.03, x,y,z=-30,20,10), right putamen (t(10)=5.75, x,y,z=27,-19,1), right frontal operculum (t(10)=8.22; x,y,z=36,17,13), and right inferior frontal gyrus (coronal image; BA 45, t(10)=5.79, x,y,z=54,5,25); and c) treatment interaction (DCS > placebo); anterior cingulate (1st coronal image; BA 24, t(21)=3.98, x,y,z=12,14,34), left insula (2nd coronal image; t(21)=4.15, x,y,z=-39,-7,10), right primary motor area (2nd coronal image, BA 4, x,y,z=39,-7,52), left dlPFC (axial image; BA 46, t(21)=4.00, x,y,z=-48,23,32) and left inferior frontal gyrus (axial image; BA 44, t(21)=4.47, x,y,z=-48,23,26).

a) Placebo-treated control group, spiders > butterflies



b) DCS-treated control group, spiders > butterflies



c) Treatment group (DCS > placebo) X Condition (spiders > butterflies) interaction

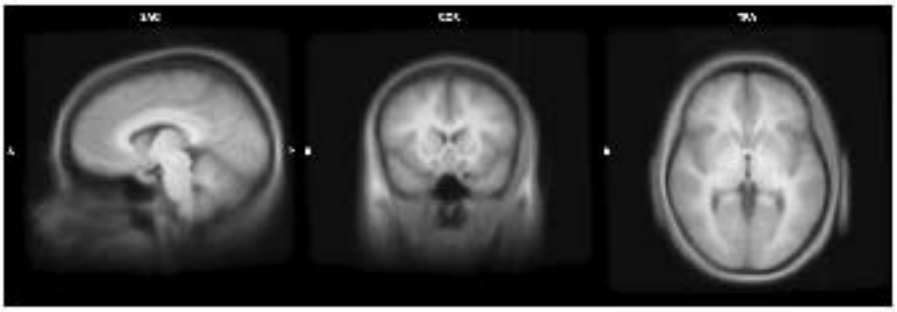


Figure 6. Regional activations for the spiders > butterflies contrast in: a) placebo-treated control group; left lateral amygdala (coronal image, t(11)=-36,29,-11; x,y,z=-27,-4,-20) and lateral frontal cortex (sagittal image; BA 4; t(11)= -5.05, x,y,z=-24,-16,52), b) DCS-treated control goup: left frontal operculum (sagittal image; t(11)=5.64, x,y,z=-39,20,10), and medial superior rostral gyrus (transverse/axial image; BA 10, t(11)=6.39, x,y,z=-6,56,4), and c) treatment (DCS > placebo) interaction; ventral ACC (sagittal and axial image; BA 24; t(21)= 3.916084, x,y,z = -6,26,4), left caudate (coronal image; t(21)=4.61; x,y,z=-9,14,10).

List of Appendices

Appendix A.

Risks/benefit analysis and data and safety monitoring plan submitted to the KUMC Institutional Review Board.

Risks and Benefits of the Proposed Study

This study is considered to involve moderate risk, as it involves 1) a study drug and 2) research associated procedures (MRI and the BAT assessment) with a low frequency of serious adverse events. As described above, DCS has been shown through 30 years of research and clinical treatment with tuberculosis, to be safe for human use (Heresco-Levy et al., 2002). The following side effects of daily, prolonged administration of DCS (for 1 week or more) have been reported: tremor, hallucinations, delusions, catatonic reactions, clinical depression, exaggerated reflexes, speech difficulties (dysarthria), and slight paralysis (Cascella et al., 1994; Mandell & Sande, 1990; Storey & McLean, 1957). Additionally, there is some indication that epilepsy patients may be at higher risk of developing side effects from repeated administration with DCS (Wlaz et al., 1994). No drug-related side effects have been reported in studies using acute administration with a single dose of 250 mg DCS. Exclusion criteria implemented in the proposed study (describe above) will serve to further reduce the risk of side effects. DCS administration will be monitored by nursing staff at the General Clinical Research Center (GCRC) of KUMC 2-3 hours after administration. The medical monitor (Sharon Cain, M.D.) and Sharon Cain, M.D. will be available for consultation should side effects or adverse events occur. If medically necessary, Dr. Hall or KUMC investigational pharmacy staff, under the direction of the medical monitor (Sharon Cain, M.D.) or Cary Savage, Ph.D. can break this blind and inform research personnel of the group assignment of a particular subject.

Participants will also be asked to contact Cary Savage, Ph.D. if they experience any side effects within one week following participation.

The MR imaging procedure does not pose a significant risk to subjects meeting the entry criteria for this study. Some subjects may experience mild claustrophobia during MRI. Also, the MRI unit makes loud noises during the examination. To minimize any possible discomfort from these, participants will be given earplugs and ear phones. Although participants are usually left alone in the MRI suite during scanning, research personnel will stay if requested by the participant. To minimize the occurrence of claustrophobic symptoms, participants identified during initial assessment (using the ADIS) as having claustrophobia will be excluded from the study. Every effort will be made to reassure the patient and minimize any discomforts.

Many of the assessments and tasks involved in this study will require participants to look at pictures of spiders, be in the same room as a live spider, and answer questions regarding their thoughts and feelings about spiders. This may cause the participants with spider phobia to experience discomfort. However, these tasks are not dangerous to the participant's physical or emotional health. Lisa Hale, Ph.D. and Sharon Cain, M.D. will be available for consultation during the administration of DCS and throughout the fMRI and testing procedures to be consulted in case of psychological or psychiatric complications. Through the behavioral avoidance task, subjects will be given the opportunity to touch and/or hold the Chilean rose hair tarantula used in the study. *However, subjects will never be forced to touch or hold the spider*. Chilean rose hair tarantulas, hereafter referred to as CHR tarantulas, were chosen for the current study based on their docile nature and intimidating appearance. Because of the location of its

fangs, a tarantula must raise itself on its hind legs to inflict a bite. However, tarantulas rarely inflict bites on humans (Diaz, 2004). It has been estimated that only 1 in 100,000 CHR tarantulas will actually bite humans (Schultz, 2005). Bites from the tarantula, which are as painful as a bee sting, are relatively innocuous and result in a low-grade histamine reaction (Lewis et al, 2006). This may cause irritation, mild to severe itching, swelling and redness. There is a very small chance that, if a subject or research personnel experienced an allergic reaction to a tarantula bite, anaphylaxis could follow (Fell & Kukula, 2005). Additionally, the tarantula has very fine, fiberglasslike, sharp, barbed hairs on its abdomen. There are approximately 10,000 hairs per square millimeter of abdomen. The CHR tarantula has Type III hairs which can penetrate up to 2 mm, causing local inflammation. When a tarantula is handled, it releases these hairs which can cause skin, eye and respiratory tract irritation. The dermatologic effect is a local urticarial reaction. These hairs may also become imbedded in the handler's eye. If imbedded in the conjunctiva, conjunctival infection results. The hairs can also become imbedded in the cornea and may penetrate the cornea to cause anterior chamber inflammation. Rarely, this inflammation lasts up to six years. Inhalation of these hairs may cause significant allergic rhinitis, but this is uncommon (Fell & Kukula, 2005). In a combined retrospective and nested prospective study of spider bites, a case series of nine tarantula bites in humans was described. In all of these nine instances, only mild effects occurred, including local pain (severe in four cases), puncture marks, and transient bleeding from the puncture site. Mild systemic toxicity occurred in one of the cases. There have been no reports of a human dying as a result of a tarantula bite or through contact with a tarantula (Isbister et

al., 2003 as reported in Diaz, 2004; Schultz, 2005). Further, our research team's consultation with other laboratories in the US, Canada, and Europe that use live spider stimuli yielded no reported occurrences of bites nor other recommended precautions (Viz. Lars-Göran Öst, Ph.D. Department of Psychology, Stockholms Universitet, Sweden; Michelle Craske, PhD and Jayson Mystkowski, Ph.D., UCLA Anxiety Disorders Behavioral Research Program. Dr. Ost originated the protocols for spider phobia treatments commonly used today, and Drs. Craske & Mystkowski, have directed more than ten years of studies utilizing exposure tasks with tarantulas).

Should a study subject come in contact with the tarantula, they will be asked to keep the tarantula far away from their eyes and face and will additionally be asked to subsequently wash their hands with soap and water. Research personnel who will be caring for and feeding the tarantula and may therefore come in contact with the CHR tarantula, will be required to sign a risk information form, recognizing that they are aware of the risks involved in handling the spider and the precautions that should be taken (see Appendix J). Should a tarantula bite occur, the puncture will be cleaned with soap and water and the subject will be offered a bandage. If it is suspected that a subject or research personnel are experiencing any symptom associated with a mild allergic reaction to a tarantula bite or the CHR abdominal hairs, the individual will be offered an oral dose of Benadryl and the study medical monitor will be consulted. If the medical monitor judges the reaction to require additional medical attention, the appropriate medical personnel will be consulted. An Ophthalmologist will be consulted when it is suspected that tarantula hairs have become imbedded in one's eye. Dr. Nathan Culley provided a

basic husbandry SOP for spiders, which will be used to direct research personnel regarding the care of the tarantula (Appendix K).

The risks associated with the behavioral and cognitive tests, magnetic resonance imaging (MRI), and the single 250 mg dose of DCS, are minimal and serious adverse events are not expected (as discussed previously). If an adverse event should occur (either serious or non-serious), the participant will, of course, have the opportunity to withdraw from the study. Additionally, the medical monitor (Sharon Cain, M.D.) and Lisa Hale, Ph.D. will be available for constulation should an adverse event occur. If any of these health practitioners believe it to be in the participant's best interest to withdraw from the study, participation of that individual will be terminated.

Results from the current study will provide valuable information as to the effects of DCS on brain reactivity and cognitive functioning in spider phobia and healthy controls. This will inform future clinical research about the mechanisms involved in DCS-supplemented exposure therapy. and could lead to more effective treatments for phobia and other anxiety disorders. It is believed that the minimal risk involved in the proposed study is reasonable considering that it could lead to more effective treatments for phobia and other anxiety disorders.

Data and Safety Monitoring: The intial assessment and all behavioral and cognitive assessments will be completed in a private room with only the examiner present. If there is reason for additional research staff to be present or to observe such assessments (e.g. for research personnel training), the participant will be asked for their verbal consent. The MRI control room and scanner is located in a private area. The door to the control room

will be locked during scanning to prevent individuals not involved in the study from entering. Only research personnel and MRI technicians will be allowed within the scanning room when the participant is being scanned. If the participant wishes for family members or friends to observe the MRI scan, these individuals will also be allowed in the control room.

Sharon Cain, M.D. will serve as the medical monitor and Jeff Burns, M.D. will serve as the safety monitor for the proposed study. Throughout the study, research personnel will be monitoring for issues related to safety of participants and personnel as well as privacy of data. When issues arise related to medical safety, the medical monitor will be contacted for assistance. Should an adverse event occur, it will be documented by research personnel. After such an event, the situation will be reviewed by a safety monitor that is independent of the study (Jeff Burns, M.D.), who will make recommendations regarding any changes in the protocol, consent form, or other aspect of the study. While the safety monitor is reviewing the adverse event, no additional data will be collected. The following information will be monitored throughout the study: number of subjects screened and enrolled, drop-outs, primary and secondary efficacy endpoints, and serious and non-serious adverse events. This data, with the exception of serious adverse events, will be reported every 12 months, coinciding with HSC periodic review. Because no serious adverse events are anticipated in this study, any such events will be immediately reported to the KUMC Institutional Review Board and the GCRC and appropriate changes to the study or consent form will be completed. Serious adverse events will be reported to the KUMC Institutional Review Board and GCRC using the

Common Toxicity Criteria (CTC)-III scale for categorization and classification of adverse events. If the severity of an adverse event requires emergency medical attention, appropriate KUMC providers/staff will be contacted to provide medical attention. However, as the University of Kansas Medical Center does not provide free medical treatment or other forms of compensation to persons injured as a result of participating in research, the participant will be billed for such medical care. Non-serious *anticipated* events will not be reported to the IRB whereas non-serious, *unanticipated* events will be reported every 12 months, as described.

Identification numbers will be assigned to each subject at the time of the initial assessment. DCS and placebo will be obtained from and prepared by the KUMC investigational pharmacy. Medications will be administered at the beginning of the appointment by the KUMC General Clinical Research Center (GCRC). Randomization of participants to receive either DCS or placebo will be conducted by Sandra Hall, Ph.D. (Research Assistant Professor, Department of Preventive Medicine and Public Health, KUMC), who will communicate the results of randomization (subject numbers and group assignment of each) to staff at the KUMC investigational pharmacy. This is to ensure that investigators, test administrators, and participants will be blind to group assignment. Dr. Hall will conduct separate, block randomizations for the phobic and non-phobic groups, so that half of each group will be randomized to receive DCS while the other half is randomized to receive placebo. Each subject will therefore have a 50% chance of receiving DCS. The list of subject ID numbers and corresponding randomization will be provided in a sealed envelope to the KUMC investigational pharmacy. Staff at the investigational pharmacy will find the

appropriate ID number for each subject as they come in and determine from the randomization list whether to give the subject drug or placebo. If medically necessary, Dr. Hall or KUMC investigational pharmacy staff, under the direction of Cary Savage, Ph.D. or Sharon Cain, M.D., can break this blind and inform research personnel of the group assignment of a particular subject.

All other data collected through the duration of the study will be kept in locked cabinets within HBIC. Data used for analysis will be de-identified and accessible by only research personnel. The staff of the GCRC will have access to information regarding demographic and medical information (as collected through the eligibility survey, Appendix A) and identification number each subject. After the data is initially entered by a member of the research team, an additional member will review the data and identify errors. A log will be kept of all data collection and analysis steps completed for each subject. For the MRI scans, a research log will be kept by the lab technicians regarding steps completed and any errors detected. MRI data will be saved on a CD kept by the lab technicians, as well as a CD kept by research personnel. Behavioral and cognitive data will be saved to a hard drive and backed up on a CD kept by research personnel. The CDs used by research personnel will also be kept in a locked cabinet at HBIC.

Appendix B.

Tables displaying complete results for each statistical contrast conducted for the fMRI symptom provocation paradigm.

Table 1.

Placebo phobics; Spiders > Butterflies, RFX .001

Side	Region	# voxels	Max T	x	y	z
right	vlPFC (BA 45/46)	91	8.22	45	32	10
right	dlPFC (BA 9)	28	7.35	42	5	37
left	lateral frontal (BA 4)	3	-5.05	-24	-16	52
right	lateral PFC (BA 6)	7	5.21	9	2	55
right	lateral frontal (BA4)	4	-5.04	48	-16	31
left	frontal operculum	4	-5.49	-48	-13	16
left	frontal operculum	7	5.23	-45	23	7
right	frontal operculum	15	5.47	45	2	1
right	insula	6	5.41	36	2	1
right	posterior insula	61	-9.60	33	-16	13
right	compact insular claustrum	5	5.71	30	11	1
left	limitans claustrum	17	6.88	-33	-13	-5
left	lateral amygdala	531	9.06	-24	-4	-14
	(cluster also included basolateral, central, and basomedial amygdala)					
right	basomedial amygdala	45	5.97	18	-1	-11
right	central amygdala	4	4.72	24	-4	-11
right	ventral pallidum	5	4.93	12	-1	-5
right	CA1 field of the hippocampus	35	6.37	27	-10	-14
left	CA3 field of hippocampus	10	5.56	-24	-19	-14
left	ventral putamen	8	5.33	-30	-22	-5
left	cerebral peduncle	169	6.14	-12	-13	-14
right	mammilary nucleus	75	6.68	6	-10	-11
left	central tegmental tract	212	5.86	-3	-28	-5
right	dorsal hypothalamus	10	5.83	6	-7	-2
left	fusiform gyrus (BA 19)	4	4.70	-21	-64	-14
right	fusiform gyrus (BA 19)	62	7.51	24	-58	-14
left	inferior temporal (BA 37)	15	5.	-45	-70	1
left	anterior transverse temporal gyrus	77	-7.57	-51	-13	4
right	planum temporale (BA 40)	9	5.00	60	-37	28
right	medial temporal gyrus (BA 19)	451	10.40	48	-67	-2
right	dorsal temporopolar (BA 28)	32	7.41	36	11	-20
left	inferior temporal gyrus (BA 21)	6	5.36	-39	2	-35
right	occipital (BA 19)	9	5.20	39	-73	1
left	occipital (BA 19)	65	6.22	-42	-64	-14
left	occpital gyrus (BA 19)	4	5.33	-30	-73	-17
mid	lingual gyrus (BA 18)	69	-6.61	3	-70	4
left	occipital gyrus (BA 18)	56	5.70	-24	-67	-17
right	parietal operculum	8	-4.92	54	-13	13
right	cerebellum	81	6.47	37	-55	-28
left	cerebellum	22	6.03	-39	-61	-23
left	cerebellum	173	6.71	-24	-31	-20

Abbreviations: BA=Brodmann area; PFC=prefrontal cortex; dlPFC=dorsolateral PFC; vlPFC=ventrolateral PFC.

Appendix B. (cont.)

Table 2.

Placebo controls: Spiders > Butterflies, RFX .001

Side	Region	# voxels	Max T	x	y	z
right	inferior frontal gyrus (BA 45)	7	5.59	45	-1	31
right	lateral frontal (BA 4)	28	6.24	18	-16	61
left	OFC (BA 47)	6	5.37	-30	32	-5
left	superior frontopolar gyrus (BA 10)	6	-5.40	-18	59	1
left	frontal operculum	5	5.33	-45	2	1
right	insula	3	5.43	39	-7	1
right	insula	11	5.12	36	2	1
right	preamygdalar claustrum	16	5.32	27	-1	-11
left	lateral amygdala	20	5.30	-27	-4	-20
right	fusiform gyrus (BA 37)	40	6.80	27	-43	-17
left	fusiform gyrus (BA 37)	14	6.30	-24	-43	-17
left	fusiform gyrus (BA 19)	63	6.08	-39	-67	-14
right	fusiform gyrus (BA 36)	18	7.34	36	-34	-20
right	cerebral peduncle	17	5.55	15	-16	-5
left	dorsal trigeminal-thalamic tract	4	4.69	-6	-28	-5
left	temporal gyrus (BA 37)	140	6.73	-51	-61	-7
right	superior temporal gyrus (BA 39)	10	-5.10	45	-67	31
right	medial temporal gyrus (BA 21)	43	7.23	45	-43	1
right	inferior temporal gyrus (BA 19)	1780	9.86	48	-64	-2
left	inferior temporal gyrus (BA 20)	4	4.97	-54	2	-20
right	inferior temporal gyrus (BA 37)	35	5.62	42	-40	-14
right	occipital gyrus (BA 19)	82	5.82	45	-70	7
left	occipital gyrus (BA 19)	44	6.57	-54	-64	-5
left	occpital gyrus (BA 37)	4	4.67	-45	-70	4
left	lingual gyrus (BA 31)	5	-5.28	-6	-70	10
right	cerebellum	5	4.73	39	-64	-23
left	cerebellum	451	9.02	-33	-37	-23

Abbreviations: BA=Brodmann area; OFC=orbitofrontal cortex.

Appendix B. (cont.)

Table 3.

Placebo phobic > placebo control; Spiders > Butterflies, RFX .001

Side	Region	# voxels	Max T	x	y	z
right	lateral frontal (BA 3/4)	30	-5.02	48	-16	31
right	lateral parietal (BA 1/3)	58	-4.88	45	-19	49
left	lateral parietal (BA 1/3)	114	-4.91	-55	-16	46
left	dlPFC (BA 9)	15	-4.54	-48	23	31
right	frontal operculum	8	-4.21	48	-16	19
left	frontal operculum	10	-4.41	-45	-13	16
right	insula	9	-4.70	33	-16	13
left	putamen	4	4.56	-24	-7	1
right	parahippocampal gyrus	16	-4.80	24	-25	-20
right	bed nucleus of the stria terminalis	4	4.42	9	-1	-2
right	parietal operculum	3	-3.97	48	-22	19
left	inferior parietal lobe (BA 40)	63	-4.46	-36	-43	58
left	temporal cortex (BA 7)	3	-4.19	-51	-61	7
mid	precuneus (BA 7)	7	-4.49	3	-49	58
right	cerebellum	19	4.57	33	-58	-38
right	cerebellum	3	4.03	22	-25	-38
mid	cerebellum	19	4.11	3	-46	-38

Abbreviations: BA=Brodmann area; PFC=prefrontal cortex; dlPFC=dorsolateral PFC.

Appendix B. (cont.)

Table 4.

Placebo phobic > placebo control; Spiders > low-level baseline, RFX .001

Side	Region	# voxels	Max T	x	y	z
right	lateral PFC (BA 6)	7	-4.68	60	5	28
right	lateral PFC (BA 6)	23	5.14	39	-7	43
mid	cingulate (BA 24)	15	4.24	0	5	40
right	frontal operculum	72	5.26	45	14	-2
right	frontal operculum	14	4.70	45	-1	1
right	frontal operculum	30	4.31	36	20	1
left	frontal operculum	3	4.21	-42	11	7
left	insula	15	4.72	-27	17	-5
left	insula	58	4.39	-30	17	10
right	insula	114	6.03	39	-10	-8
left	medial caudate	13 (+6)	4.48	-15	5	1
right	medial caudate	3	4.07	12	11	10
left	putamen	7 (+5)	4.13	-24	-4	1
mid	pons	148	5.61	0	-19	-17
right	bed nucleus of the stria terminalis	15	4.88	9	-1	-2
left	cerebral peduncle	5	4.35	-18	-19	-5
left	cucullaris nucleus	144	4.68	-3	-16	13
mid	superior cerebellar peduncle	4	4.25	3	-31	-14
left	dorsal temporopolar (BA 38)	3	4.04	-39	11	-14
right	inferior parietal lobe (BA 40)	13	4.24	57	-40	28
left	cerebellum	26	4.60	-24	-37	-23
right	cerebellum	16	4.22	9	-43	-17

Abbreviations: BA=Brodmann area; PFC=prefrontal cortex.

Appendix B. (cont.)

Table 5.

Placebo phobic > placebo control; Butterflies > low-level baseline, RFX .001

Side	Region	# voxels	Max T	x	y	z
left	lateral PFC (BA 6)	18	4.84	-15	-16	70
mid	superior PFC (BA 6)	25	4.56	3	-10	55
right	lateral parietal (BA 2)	21	4.92	54	-19	31
left	lateral parietal (BA 3)	10	4.61	-24	-31	67
right	lateral frontal (BA 4)	4	-4.65	54	-10	25
left	lateral frontal (BA 4)	17	4.78	-30	-22	64
right	lateral frontal (BA 4)	12	4.45	24	-19	55
mid	posterior cingulate (BA 31)	33	4.64	-3	-31	34
left	anterior cingulate (BA 24/32)	43	4.67	-6	29	10
right	posterior cingulate (BA 24)	26	5.24	12	-16	37
left	isthmus of cingulate gyrus	33	5.28	-9	-49	4
right	frontal operculum	29	4.58	42	-4	4
left	frontal operculum	11	4.24	-33	14	16
left	insula	54	5.32	-36	-25	10
right	insula	26	5.32	42	-13	7
right	insula	7	3.98	39	-7	10
left	insula	15	4.38	-39	-4	10
left	medial caudate	7	4.35	-24	8	7
right	putamen	17	4.75	15	14	1
mid	fornix	42	4.79	-3	-4	1
right	perirhinal cortex	40	4.81	27	-7	-23
left	inferior temporal (BA 21)	19	4.67	-42	8	-32
right	superior temporal (BA 22/42)	25	4.64	48	-6	7
right	superior temporal (BA 22)	4	4.45	51	5	-5
right	superior temporal (BA 42)	242	5.26	54	-28	13
left	parietal (BA 7/40)	5	4.29	-27	-40	52
right	inferior parietal lobe (BA 40)	66	4.50	53	-37	28
right	superior parietal lobe (BA 7)	19	5.24	24	-46	61
left	occipital (BA 19)	16	4.30	-33	-67	34
left	cerebellum	6	4.37	-21	-28	-41
left	cerebellum	17	4.14	-9	-61	-23
right	cerebellum	16	4.19	9	-61	-23
right	cerebellum	29	4.83	15	-58	-23
left	cerebellum	35	5.33	-6	-61	-8

Abbreviations: BA=Brodmann area; PFC=prefrontal cortex.

Appendix B. (cont.)

Table 6.

DCS-treated phobics: Spiders > Butterflies, RFX .001

Side	Region	# voxels	Max T	x	y	z
mid	superior PFC (BA 6)	5	4.971816	-3	-7	64
left	lateral PFC (BA 6)	5	5.367335	-9	23	55
left	lateral PFC (BA 6)	78	7.883197	-15	-7	61
left	lateral PFC (BA 6)	3	5.149535	-18	-16	64
right	lateral PFC (BA 6)	3	4.943971	45	-7	37
left	lateral PFC (BA 4/6)	278	8.029253	-48	-4	40
right	lateral frontal (BA 4)	531	10.307141	36	-7	49
left	inferior frontal (BA 45)	9	5.214232	-45	20	22
right	inferior frontal (BA 44)	5	4.892351	60	11	16
right	inferior frontal (BA 45)	35	5.786233	54	5	25
right	inferior frontal (BA 44)	12	5.168444	45	8	28
left	dlPFC (BA 9)	3	5.321159	-33	35	34
right	dlPFC (BA 9/46)	41	5.678327	36	29	31
left	dlPFC (BA 9/46)	760	7.998677	-24	44	28
right	dlPFC (BA 46)	5	5.282866	39	41	19
right	dlPFC (BA 46)	8	5.603292	45	35	13
right	dlPFC (BA 46)	19	5.468701	42	41	14
right	inferior OFC (BA 47)	9	5.206333	45	17	-5
right	inferior OFC (BA 47)	98	5.874109	39	26	1
left	lateral orbital gyrus	14	4.995579	-33	35	-11
right	dorsal cingulate (BA 32)	6	5.155251	6	17	31
mid	dorsal cingulate (BA 32)	213	6.776052	-3	17	31
right	ventral cingulate (BA 24)	2922	8.786025	6	2	46
right	frontal operculum	167	5.707574	46	5	4
right	frontal operculum	3	5.273075	39	-1	10
right	frontal operculum	313	8.216325	36	17	13
right	frontal operculum	3	4.718333	48	14	-2
left	frontal operculum	20	5.473941	-45	2	1
left	insula	299	8.026821	-30	20	10
right	insula	124	6.676866	36	2	4
right	caudate and internal capsule	637	9.666705	15	-10	13
left	dorsal claustrum	7	4.928164	-33	-13	-2
right	putamen	50	5.75498	27	-19	1
right	medial putamen	46	6.5412	24	5	7
right	parahippocampal gyrus	52	6.204882	24	-43	-8
mid	precuneus	4	-4.73595	3	-61	22
right	fusiform gyrus (BA 36)	1238	8.502882	39	-52	-20
right	fusiform gyrus (BA 36)	75	6.894574	33	-13	-26
left	fusiform gyrus (BA 36/37)	499	7.572194	-24	-40	-17
left	fusiform gyrus (BA 20)	3	4.924512	-27	-4	-32
left	inferior temporal gyrus (BA 37)	1293	7.727646	-42	-58	-11
left	inferior temporal gyrus (BA 37)	15	5.092521	-39	-46	-17
right	inferior temporal (BA 19)	243	6.74443	39	-58	-2
right	superior temporal gyrus (BA 22)	4	5.03045	51	5	-2
left	angular gyrus (BA 19)	34	5.571859	-27	-61	43
right	angular gyrus (BA 40)	6	5.009749	33	-52	46
right	superior parietal lobe (BA 19)	191	6.539726	21	-67	43

Appendix B. (cont.)

Table 6 (cont.).

DCS-treated Phobics: Spiders > Butterflies, RFX .001

Side	Region	# voxels	Max T	x	y	z
left	superior parietal lobe (BA 7)	9	6.184313	-15	-55	55
left	medial geniculate nucleus	124	5.763817	-18	-28	-5
left	ventroanterior thalamus	47	5.80396	-9	-10	10
left	ventrolateral posterior thalamus	9	5.572302	-12	-22	4
right	right inferior pulvinar nucleus	4	4.820837	21	-31	10
left	inferior pulvinar nucleus	41	6.187953	-21	-31	10
mid	central tegmental tract	55	6.657354	-3	-28	-5
right	red nucleus, parvocellular part	139	10.242029	6	-22	-5
right	cerebral peduncle	3	5.275124	6	-16	-14
right	cerebral peduncle	28	5.808577	15	-22	-8
right	pons	13	5.157822	6	-22	-17
left	occipital gyrus (BA 18/31)	4	4.718277	-21	-70	22
left	lingual gyrus (BA 19)	25	6.883592	-21	-58	-5
right	occipital (BA 19)	42	5.310084	45	-73	10
right	occipital gyrus (BA 19/37)	210	7.604238	42	-64	-17
left	sagittal striatum	4	4.665262	-24	-76	7
right	occipital gyrus (BA 19)	525	8.428448	36	-70	-2
right	cerebellum	96	7.718362	33	-61	-26
right	cerebellum	19	5.507664	30	-40	-23
right	cerebellum	8	5.545151	33	-70	-32
right	cerebellum	117	5.17111	21	-70	-20
right	cerebellum	117	6.446866	9	-43	-17
right	cerebellum	23	5.901428	6	-61	-35
mid	cerebellum	27	5.135165	0	-52	-35
left	cerebellum	10	5.249269	-3	-43	-20
left	cerebellum	9	5.024959	-6	-70	-23
left	cerebellum	30	5.441673	-21	-67	-20
left	cerebellum	176	6.526022	-33	-55	-29

Abbreviations: BA=Brodmann area; PFC=prefrontal cortex; dlPFC=dorsolateral PFC; OFC=orbitofrontal cortex.

Appendix B. (cont.)

Table 7.

DCS-treated controls; Spiders > Butterflies, RFX .001

Side	Region	# voxels	Max T	x	y	z
L/mid	superior rostral gyrus (BA 10)	298	6.39	-6	56	4
left	vlPFC (BA 45/46)	9	5.609	-48	26	7
left	lateral PFC (BA 6)	9	5.369	-9	53	13
left	lateral PFC (BA 6)	15	5.089	-9	5	67
left	dlPFC (BA 9)	25	5.293	-42	5	43
left	dlPFC (BA 9)	5	5.209	-6	50	34
left	lateral OFC	21	5.12	-36	29	-11
mid	posterior cingulate (BA 23)	7	4.80	-3	-22	34
left	anterior cingulate (BA 32)	40	6.90	-15	44	10
left	frontal operculum	30	5.64	-39	20	10
left	parahippocampal gyrus	17	5.23	-24	-34	-14
left	CA1 field of hippocampus	7	-4.85	-30	-40	-2
right	piriform cortex	6	5.33	27	11	-11
left	uncinate fasciculus	3	5.20	-30	5	-5
right	fusiform gyrus (BA 37)	59	6.69	45	-52	-20
left	fusiform gyrus (BA 37)	28	6.16	-39	-49	-14
right	inferior temporal (BA 19)	15	5.51	45	-67	-2
left	temporal gyrus (BA 37)	5	4.91	-51	-61	10
right	superior temporal gyrus (BA 37/21)	49	5.68	42	-58	4
left	superior temporal gyrus (BA 38)	7	5.52	-48	11	-20
left	temporal gyrus (BA 39)	3	4.85	-51	-70	13
left	occipital gyrus (BA 19)	7	5.54	-33	-73	10
right	cerebellum	9	-5.45	28	-37	-38
mid	cerebellum	8	-4.72	-2	-49	-10

Abbreviations: BA=Brodmann area; PFC=prefrontal cortex; dlPFC=dorsolateral PFC; vlPFC=ventrolateral PFC.

Appendix B. (cont.)

Table 8.

DCS-treated phobics > Placebo-treated phobics; Spiders > Butterflies; RFX .001

Side	Region	# voxels	Max T	x	y	z
left	dlPFC (BA 9)	5	4.41	-36	29	31
left	dlPFC (BA 46)	4	4.00	-48	23	32
left	inferior frontal (BA 44)	41	4.47	-48	23	26
right	inferior frontal (BA 45)	16	4.66	48	8	28
right	inferior frontal (BA 45)	9	4.24	36	-13	46
right	lateral frontal (BA 4)	14	4.39	48	-13	31
right	lateral frontal (BA 4)	15	4.46	39	-7	52
right	lateral PFC (BA 6)	6	4.23	3	-1	58
right	cingulate (BA 24)	6	3.98	12	14	34
right	insula	4	3.86	33	-16	13
left	insula	47	6.19	-39	-7	10
left	insula	4	4.15	-39	-16	-5
right	zona incerta	23	4.13	15	-19	-2
right	fusiform gyrus (BA 36)	29	5.27	34	-13	-26
right	inferior temporal gyrus (BA 19)	22	4.27	45	-61	-2
left	superior temporal (BA 21)	12	4.19	-45	-43	10
left	temporal cortex (BA 37)	16	4.16	-42	-58	4
left	superior parietal lobe (BA 7)	25	4.64	-27	-58	52
right	lingual gyrus (BA 18)	28	4.32	15	-67	-11
right	lingual gyrus (BA 19)	173	5.22	15	-58	1
left	lingual gyrus (BA 19)	25	4.68	-21	-58	-5
right	occipital cortex (BA 37/19)	171	7.56	33	-70	1
right	cerebellum	4	3.84	9	-61	-23
left	cerebellum	9	4.03	-6	-67	-41
mid	cerebelum	96	4.84	0	-67	-20

Abbreviations: BA=Brodmann area; PFC=prefrontal cortex; dlPFC=dorsolateral PFC.

Appendix B. (cont.)

Table 9.

DCS-treated phobics > placebo-treated phobics; Spiders > low-level baseline, RFX .001

Side	Region	# voxels	Max T	x	y	z
left	inferior frontal (BA 45)	22	4.88	-48	23	22
right	basal operculum	3	3.98	30	20	-8
right	dlPFC (BA 9/46)	4	4.14	27	41	31
left	presubiculum	23	-4.66	-18	-22	-14
right	thalamic fasciculus, field H1	20	4.38	9	-22	-2
right	fusiform gyrus (BA 36)	5	4.30	31	-16	-26
left	superior temporal (BA 37)	4	4.04	-42	-58	7
right	inferior parietal lobe (BA 40)	35	-4.71	54	-37	25
left	occipital cortex (BA 19)	11	4.20	-24	-67	28
left	cerebellum	29	4.55	-6	-64	-32

Abbreviations: BA=Brodmann area; PFC=prefrontal cortex; dlPFC=dorsolateral PFC.

Appendix B. (cont.)

Table 10.

DCS-treated phobics > placebo-treated phobics; Butterflies > low-level baseline, RFX .001

Side	Region	# voxels	Max T	x	y	z
right	lateral PFC (BA 6)	24	-4.78	12	2	58
left	lateral PFC (BA 6)	4	-3.95	-42	17	47
left	inferior frontal (BA 44)	96	-4.75	-33	1	31
left	dlPFC (BA 9)	4	-3.94	-18	35	31
left	cingulate (BA 31)	48	-5.61	-3	-31	34
right	isthmus of cingulate gyrus	3	-4.13	6	-46	10
right	subiculum	19	-4.34	24	-37	-5
left	parasubiculum	2	-4.00	-18	-13	-20
right	claustrum	9	-4.21	30	-22	4
mid	precuneus	4	-4.05	0	-58	55
left	medial caudate	7	-4.64	-24	8	7
left	ventrolateral posterior thalamus	69	-4.57	-15	-22	19
left	cerebral peduncle	8	-4.44	-15	-19	-8
left	reticular thalamic nucleus	3	-4.34	-27	-34	4
right	superior temporal (BA 22)	5	-4.23	48	-49	16
left	lingual gyrus	126	-5.50	-21	-52	1
left	internal sagittal stratum	32	-4.62	-39	-46	1

Abbreviations: BA = Brodmann area; PFC=prefrontal cortex; dlPFC—dorsolateral PFC.

Appendix B. (cont.)

Table 11.

DCS-treated controls> placebo-treated controls, RFX .001

Side	Region	# voxels	Max T	x	y	z
Spiders > Butterflies						
left	ventral ACC (BA 24)	6	3.92	-6	26	4
left	medial caudate	3	4.61	-9	14	10
left	parietal cortex (BA3)	11	-4.70	-30	-31	55
right	fusiform gyrus (BA 37)	39	-4.91	42	-58	-14
left	inferior temporal gyrus (BA 20)	4	4.17	-45	-7	-20
right	cerebellum	31	4.43	45	-52	-41
Spiders only						
right	lateral PFC (BA 6)	33	5.11	43	5	52
right	lateral PFC (BA 6)	5	4.17	30	-7	43
left	lateral PFC (BA 6)	25	4.40	-6	-10	58
right	lateral PFC (BA 6)	10	4.13	9	8	68
left	frontal operculum	3	3.92	-42	11	7
right	lateral parietal (BA 2)	10	4.47	54	-19	31
left	superior cerebellar peduncle	5	4.23	-3	-31	-14
left	cerebellum	9	4.11	-45	-45	-38
right	cerebellum	8	4.28	39	-49	-41
Butterflies only						
left	insula	3	3.99	-33	13	10
left	frontal operculum	4	4.58	-36	14	16
left	lateral caudate	6	4.12	-18	-1	22
left	putamen	4	4.45	-24	2	16
right	piriform cortex	10	4.48	27	2	-11
right	subcallosul stratum	21	4.21	18	-1	25
right	inferior parietal (BA 40)	29	4.91	54	-19	28
right	superior parietal (BA 7)	18	4.65	25	-46	61
left	precuneaus	26	4.31	-6	-34	49
left	cerebellum	26	4.66	-6	-4	-38

Abbreviations: BA = Brodmann area; PFC=prefrontal cortex; ACC=anterior cingulate cortex.