Fear conditioning to socially relevant stimuli in social anxiety

Ph.D. Dissertation

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To Marisol: for being my bright light and my guide whenever I needed it.

The happiest of people do not necessarily have the best of everything; they just make the most of everything that comes along their way.

(Karen S. Magee)

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Abbreviations

ADs	Anxiety Disorders
BDI	Beck Depression Inventory
BOLD	Blood-oxygen-level-dependant
BP	Blood pressure
CR	Conditioned Response
CS	Conditioned Stimulus
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorder Revised
ECA	Epidemiological Catchment Area
EMG	Electromiography
ESEMeD	European Study of Epidemiology of Mental Disorders
FC	Fear conditioning
fMRI	Functional magnetic resonance imaging
FNES	Fear of Negative Evaluation Scale
FPS	Fear Potentiated Startle
GAD	Generalized Anxiety Disorder
HC	Healthy controls
HR	Heart rate
ITI	Inter trial interval
LSAS-SR	Liebowitz Social Anxiety Scale self report version
MINI	Mini International Neuropsychiatric Scale
OCD	Obsessive Compulsive Disorder
PD	Panic Disorder
PDSS-SR	Panic Disorder Severity Scale self report version

PTSD	Posttraumatic Stress Disorder
SAD	Social Anxiety Disorder
SCR	Skin Conductance Response
SSA	Subclinical Social Anxiety
SSRI	Selective Serotonin Reuptake Inhibitors
STAI	State-Trait Anxiety Inventory
US	Unconditioned stimulus
WHO	World Health Organization

INTRODUCTION

Anxiety Disorders: what are they and where do they come from.

1. Anxiety Disorders: what are they and where do they come from

1.1 What are anxiety disorders?

Anxiety disorders (ADs) are conceptualized as disruptions of emotional processing, specifically, of exaggerated propensities to respond defensively to stimuli typically perceived as mildly threatening or even innocuous (McTeague & Lang, 2012). They are characterized by excessive and irrational fear/anxiety of everyday situations that prevent individuals from leading healthy lives (American Psychological Association, 2000). In spite of the apparent differences in symptomatology, associated impairment, and natural course, all ADs share the core role of anxiety reactions and avoidance behaviors in the development and/or the expression of the illness.

1.1.2 From fear to pathological anxiety

Fear and anxiety are closely related emotional phenomena originating in evolved mammalian defense systems. Both can be viewed as a defensive response that motivates organisms to detect, react and cope with threat and danger, involving intense negative feelings and strong bodily manifestations. These defensive responses vary with the nature of the danger and depend on whether the threat is present or not. Nonetheless, despite their overlap, research during the last decade has started to unravel important differences between them. Since earlier theories, the most common way of distinguishing fear and anxiety was whether there was a clear and obvious source of danger or whether the danger was more diffuse. The former was called fear, and the latter anxiety. Recently, however, many prominent researchers have proposed

more fundamental distinctions between fear and anxiety based on clinical, ethological, and neurobiological evidence.

Fear is a life-saving emotional state that serves to anticipate and to adapt to danger (Gallagher & Holland, 1994). It is a phasic response to an imminent and identifiable object and it denotes dread of impending disaster and an intense urge to defend oneself, primarily by getting out of the situation (Öhman & Mineka, 2001). Fear involves the activation of the fight-or-flight response of the sympathetic nervous system (Barlow, 2002).

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) anxiety, denotes an "apprehensive anticipation of future danger or misfortune accompanied by a feeling of dysphoria or somatic symptoms of tension" (p.820). Anxiety, therefore, involves negative emotions and cognitions that are more oriented to the future and much more diffuse than fear. It involves negative mood, worry or anxious apprehension about possible future threat or danger, and a sense of being unable to predict or control the future threat. Physiologically anxiety often creates a state of tension and chronic overarousal but no activation of the fight-or-flight response (Barlow, 2002).

Neurally, fear and anxiety can also be differentiated. Some studies have showed that lesions in the central nucleus of the amygdala block phasic, cued-induced fear responses without affecting more sustained anxiety states associated with uncertain danger, whereas lesions of the bed nucleus of the stria terminalis have the opposite effects (Waddell, Morris, & Bouton, 2006; Sullivan et al., 2004).

However, a fundamental question yet to be unraveled is, how pathological anxiety may develop from adaptive fear states? Since early theories, a clear and distinctive difference between clinical and normal fear/anxiety include that the former is more recurrent and persistent; that its intensity is unreasonable, given the objective danger or threat; that it tends to paralyze individuals, making them helpless and unable to cope; and that it results in impeded psychosocial or physiological functioning (e.g., Lader & Marks, 1974).

1.1.3 Classification, epidemiological data and associated costs

1.1.3.1 Classification

The term “neurosis” was first introduced by the Scottish doctor William Cullen in 1769 to refer to "disorders of sense and motion" caused by a "general affection of the nervous system". It described various nervous disorders and symptoms that could not be explained physiologically. The term was, however, most influentially defined by Carl Jung and Sigmund Freud over a century later (Russon, 2003). Freud introduced ADs under the concept of “neurosis” stating that they have their origins in the frustration of basic instincts, either because of external obstacles or because of internal mental imbalance. Based on Freud (1924), neuroses were identified as disorders caused primarily by psychological causes and a distinction was made between anxiety and phobic neuroses from other neurotic disorders.

The DSM eliminated the category of “neuroses” including them under the category of “anxiety disorders”. The current DSM-IV-TR includes 8 different ADs: Generalized

Anxiety Disorder (GAD), Panic Disorder (PD) with and without agoraphobia, Agoraphobia without history of PD, Specific Phobia, Social Anxiety Disorder (SAD), Obsessive-Compulsive Disorder (OCD), Posttraumatic Stress Disorder (PTSD) and Acute Stress Disorder.

1.1.3.2 Epidemiological data

1.1.3.2.1 Prevalence

According to the National Comorbidity Survey Replication (Kessler, Chiu, Demler, & Walters, 2005), ADs are the most common mental diseases with a lifetime prevalence of approximately 28.8% in the U.S. population, with specific phobia and SAD being the most prevalent ADs, 12.5% and 12.1% respectively. In fact, according to the World Health Organization (WHO), by 2020, anxiety and depressive disorders combined will constitute the second most frequent illness (WHO, 2001).

The European Study of the Epidemiology of Mental Disorders (ESEMeD) reported a lifetime prevalence of 13.6% for any AD in Europe (Alonso et al., 2004) and a lifetime prevalence of 9.3% in Spain (Haro et al., 2006). Within ADs, the most prevalent ones in Europe are specific phobia and GAD with a lifetime prevalence of 7.7% and 2.8% respectively (Alonso et al., 2004). In Spain, specific phobia, SAD and PD are the most prevalent ADs, with a lifetime prevalence of 3.6%, 0.6% and 0.6% respectively (Haro et al., 2006).

1.1.3.2.2 Gender differences

Gender appears to play a role in ADs. Various epidemiological studies have noted that women are at greater risk than men for developing an AD. For example, in a community sample of 1079 adolescents, Lewinsohn, Gotlib, Lewinsohn, Seeley, & Allen (1998), found that girls were more likely than boys to have a current or lifetime diagnosis of an AD. Other studies have reported adolescent girls to have a greater number of worries, more separation anxiety (Campbell & Rapee, 1994; Poulton, Milne, Craske, & Menzies, 2001; Costello, Egger, & Angold, 2003), and to be six times more likely to develop GAD than adolescent boys (Bowen, Offord, & Boyle, 1990; McGee et al., 1990).

Similar evidence for gender differences exists in adult samples. Comparable to childhood epidemiological studies, women are at greater risk than men for most ADs. According to lifetime prevalence data from the National Comorbidity Survey, women are more likely than men to develop agoraphobia, PD, GAD (Kessler et al., 1994) and PTSD (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995; Kessler, Chiu, Demler, & Walters, 2005). A recent review of the literature on gender differences in fear and anxiety (McLean & Anderson, 2009) concluded that women often exhibit higher levels of anxiety across their life span than males due to the interplay of biological and environmental factors, thus contributing to increased treatment seeking behaviors and higher chances of being diagnosed with an AD. The ESEMeD project also revealed a higher prevalence in women in the majority of ADs, except for SAD where differences in gender were not significant (Haro et al, 2006).

1.1.3.2.3 Age of onset

The age of onset of mental disorders is important for a variety of clinical and research purposes. This type of information is necessary to understand the natural history of mental illnesses (Thyer, Parrish, Curtis, Nesse, & Cameron, 1985); however, age of onset information in ADs is often quite vague. Table 1 offers a summary on the age of onset of most ADs.

Table 1. Age of onset for main Anxiety Disorders according to the DSM-IV-TR.

Diagnosis	Age of Onset
Agoraphobia with or without panic attacks	Most frequently in the late teens or early 20s, but it can be later.
Social Anxiety Disorder	Late childhood or early adolescence.
Simple Phobia	Age of onset varies according to the subtype, but more often begins in childhood.
Panic Disorder	Late adolescence or early adult life, but may also occur initially in mid-adult life
Generalized Anxiety Disorder	May develop at any age, though majority of patients have an onset during their third decade.
Obsessive Compulsive Disorder	Mainly in adolescence or early adulthood, but it may start in childhood.
Posttraumatic Stress Disorder	Can occur at any age, including childhood.

1.1.3.3 Social and economical costs

Anxiety disorders can negatively impact an individual's ability to work, maintain healthy relationships, and function optimally in day-to-day activities. According to the European ESEMeD project (Alonso et al., 2004) ADs were also associated with substantial levels of disability and loss of quality of life. The ADs with the strongest

impact were PD, PTSD and SAD. Moreover, ADs have been found to be associated with significant economic costs. Apart from the high healthcare costs that anxiety patients represent, the bulk of the economic burden of ADs occurs due to costs associated with reduced working capacity or premature pension (Rice and Miller, 1998). The cost of treating ADs in the U.S.A has been estimated at $46.6 billion, accounting for 31.5% of mental illness costs (DuPont et al., 1996). In Europe, the costs associated with ADs are also very high. The German National Health Interview and Examination Survey (Jacobi et al., 2002) showed that the costs associated with ADs in Europe range from €500 to €1600 per case in 2004.

1.2 Origins of Anxiety Disorders

The underlying etiology of ADs is complex, involving the interplay between many factors. Past and current research has revealed numerous individual factors that may predict the development of ADs, namely personality traits (e.g. neuroticism, trait anxiety), neural indexes (e.g. amygdala reactivity), genetic markers (e. g. specific polymorfisms) and learning histories, among others. Altogether, these variables constitute a range of vulnerability factors in response to significant negative life events in a diathesis-stress model of psychopathology (Zvolensky, Kotov, Antipova, & Schimdt, 2005). Considering these diatheses can lead to a better understanding of the etiology and development of ADs.

1.2.1 Preparedness theory

The preparedness theory was introduced by Seligman (1971) and later extended by Öhman (1986) to respond to clinical observations that indicate a higher prevalence of specific phobias to certain types of stimuli or situations.

The concept of selective associations is the primary feature of this theory. A selective association is said to occur when organisms show superior fear conditioning (FC) with certain combinations of conditioned (CS) and unconditioned (US) stimuli for reasons other than their simple salience. These selective associations may promote a faster acquisition of the conditioned response (CR), the acquisition of a larger response, or enhanced resistance to extinction of that response.

Seligman's preparedness theory assumes that we are more likely to fear events and situations that provided threats to the survival of our ancestors, such as potentially deadly predators, heights and wide open spaces, than to fear the most frequently encountered potentially deadly objects in our contemporary environment, such as weapons or motorcycles (Öhman & Mineka, 2001). These biological stimuli involve objects or situations that have been a threat or danger to the human species throughout its evolutionary history.

The preparedness theory relied on three assumptions (Seligman, 1971). First, that phobias stem from the experience of an initially neutral stimulus in temporal contiguity with an aversive event. Second, that stimuli can be located along a continuum of preparedness for FC ranging from prepared to contraprepared. And third, CSs

believed to be highly prepared for FC are those stimuli that have possessed biological significance for "pretechnological" people (Armfield, 2006). Frequently feared stimuli such as spiders and snakes are referred to as fear-relevant and are proposed as being highly prepared for FC (McNally & Reiss, 1982). For Seligman, the number of associations between CS and US necessary for conditioning is a good operational measure to establish the preparedness continuum.

The major advantage of the preparedness theory is its ability to explain the differential associability of phobic fear responses to certain stimuli that can be viewed potentially dangerous to "pretechnological" individuals (Armfield, 2006).

1.2.2 Cognitive theories

Cognitive theorists have proposed that ADs result from distorted beliefs about the dangerousness of certain situations, sensations and/or mental events. Many studies have shown that patients with ADs over-estimate the dangerousness of various stimuli and that such over-estimates are disorder-specific, with each ADs being associated with a particular type of negative belief (Clark, 1999). There are many considerations that are worth mentioning. It is now clear that there are many people who develop an AD and then recover without any treatment. For these people, their negative thinking seems to be self-correcting. However, this self-correction does not occur in patients who present for treatment and the persistence of their fears seems strangely irrational.

Other theorists have suggested that selective attention *towards* threat cues may play a role in the maintenance of ADs by enhancing the perception of threat. For

example, some studies have shown that enhanced awareness of body cues contributes to the maintenance of PD (Ehlers & Breuer, 1992).

On the other hand, other studies have suggested that attention *away* from threat cues may play an important role in the maintenance of ADs. For example, Clark & Wells (1995) have suggested that SAD individuals tend to avoid looking at other people in a feared social situation. Reduced processing of other people would mean that individuals with SAD would have less chance to observe other people's responses in detail and, therefore, would be unlikely to collect information from other people's reactions that would help them see that they generally come across more positively than they think (Rapee & Heimberg, 1997).

1.2.3 Genetic contribution

There is some evidence that suggests that ADs are familial and heritable. For example, two twin studies attributed 30%–40% of the variance in probability to develop PD to genetic factors. The remaining variance in liability came from individual-specific environmental factors (Kendler, Neale, Kessler, Heath, & Eaves, 1993; Scherrer et al., 2000). In a meta-analysis by Hettema, Neale, & Kendler (2001), estimates of heritability were of 0.43 for PD and 0.2–0.4 for phobic disorders. In the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders, Hettema, Prescott, Myers, Neale, & Kendler (2005), evidenced a gene contribution in ADs with heritability estimates of 0.3 for PD, 0.4 for agoraphobia, 0.1 for SAD and 0.2 for specific phobias.

Family studies that included probands with PD have documented an increased

risk between 5 and 16% among relatives of affected individuals (Horwath et al., 1995; Maier, Lichtermann, Minges, Oehrlein, & Franke, 1993). In phobic disorders, family studies have also demonstrated a familial contribution. For example, Stein et al. (1998) have shown that first-degree relatives of probands with specific phobia or SAD have a six- to nine-fold increased risk of the disorder.

1.2.4 Neurobiological factors

There is a vast literature on the neurobiological variables involved in ADs. For example, it has been shown that the recurrent symptoms of ADs, such as panic attacks, insomnia, exaggerated startle, and chronic sympathetic autonomic arousal, may reflect abnormal elevated noradrenergic function (Charney, Heninger, & Breier, 1984; Charney, Woods, Goodman, & Heninger, 1987; Grillon & Morgan, 1998). However, it remains unclear whether alterations in noradrenergic function play an etiologic role in the pathogenesis of ADs, or instead reflect secondary, compensatory changes in response to dysfunctions in other systems.

Abnormalities in corticotropin-releasing hormone, a stress related hormone facilitated by the amygdala, seem also to play a role in ADs. For example, some studies have found this hormone to be abnormally increased in individuals with PTSD (Bremner et al., 1997; Baker et al., 1999; but see Pitman & Orr, 1990 or Yehuda et al., 1995).

Clinical evidence suggests that benzodiazepine BZD-receptor agonists have anxiolytic effects and that benzodiazepine BZD-receptor function may be altered in ADs. They have been implicated in ADs on the basis of the anxiolytic and anxiogenic

properties of BZD agonists and inverse agonists, respectively, and by the evidence that the BZD-receptor sensitivity to BZD agonists is reduced in patients with ADs. For example, in PD oral (Woods, Charney, Silver, Krystal, & Heninger, 1991) and intravenous (Nutt, Glue, Lawson, & Wilson, 1990) administration of the BZD-receptor antagonist, flumazenil, produces panic attacks and increases anticipatory anxiety.

Regarding the serotoninergic function in ADs, it has been reported to be abnormally elevated (Norman et al., 1986), normal (Balon, Pohl, Yeragani, Rainey, & Oxenkrug, 1987), or abnormally reduced (Pecknold, Suranyi-Cadotte, Chang, & Nair, 1988) in PD. The results are therefore inconsistent. Patients with combat-related PTSD show decreased paroxetine binding in platelets relative to healthy controls (HC), a finding suggesting alterations in the serotoninergic system (Arora, Fichtner, O'Connor, & Crayton, 1993).

1.2.5 Fear Conditioning models

Fear conditioning models have long been implicated in the etiology of ADs, although they have received much criticism since the early 1970s (Mineka & Zinbarg, 2006) because of their lack of ability to account for other factors involved in the development and maintenance of ADs (e.g. individual differences). However, there has been a reappearance of interest of these models as they have recently incorporated some of the complexity predicted by new contemporary learning approaches.

The conditioning model of ADs states that anxiety originates by simple classical conditioning (Pavlov, 1927; Watson & Rayner, 1929). Later, this model shifted toward a

model where FC played a role of motivation and reinforcement of avoidance (Eysenck, 1979). This “new” approach took into account the role of incubation of fear (Eysenck, 1985); the principle of preparedness discussed earlier (Seligman, 1971; Öhman, 1986); and the role of impaired inhibition to safety cues (Davis, Falls, & Gewirtz, 2000), stimulus generalization (Mineka & Zinbarg, 2006), and enhanced conditionability (Orr et al., 2000) in the development and maintenance of ADs.

These models have received support from at least four sources: 1) the effectiveness of exposure therapy in the treatment of ADs (Graham & Milad, 2011), 2) findings of increased anxiety in PTSD (Grillon & Morgan, 1998), 3) mixed support from retrospective accounts in clinical patients and 4) current findings of laboratory-based FC studies. Fear conditioning models of ADs will be discussed in detail in the next section.

Fear Conditioning in Anxiety Disorders

2. Fear Conditioning in Anxiety Disorders

2.1 What is Fear Conditioning?

Fear conditioning has been used for exploring the neurobiological basis of fear learning and is one of the most successful laboratory tools to study the pathogenesis of ADs. It is a form of associative learning where a neutral stimulus is repeatedly paired with an aversive US, which provokes a reaction until the neutral stimulus turns into the CS and is capable of eliciting a CR. The analogy between FC and the pathogenesis of ADs has been very important in the development of novel techniques to reduce pathological anxiety.

It can be stated that FC is an adaptive phenomenon that helps detect warning and safety signals. When detecting a warning signal, is entirely normal that an organism exhibits fear, especially when this fear will prevent it from being harmed. In fact, in laboratory-based studies almost everyone will learn to react fearfully when confronted with neutral stimulus that predicts the occurrence of an aversive outcome (Beckers, Krypotos, Bodeez, Effting, & Kindt, 2013). However, in contrast with the adaptive nature of FC, an organism with pathological anxiety will present an out-of-measure behavior, excessive avoidance and exceedingly high levels of subjective fear when confronted to this same stimulus (Barlow, 2002). Studies comparing clinical and non-clinical samples support this idea and suggest that abnormalities in FC play a major role in etiological accounts of ADs.

Despite the fact that FC serves as an adaptive, self-preserving function, it can

become a source of pathology when anxious reactivity persists to the CSs in the absence of the CS-US contingency.

2.1.1 Historical overview

Ivan Pavlov was the first to describe classical conditioning. Pavlov experimented with a dog, ringing a bell (CS) every time he fed him. After a number of pairings of the bell and the food (US), he observed that the dog salivated when the bell rang, though in absence of the food. He named this type of learning "conditioning". This type of classical conditioning became to be known as "appetitive conditioning". Fear conditioning is based on the classical conditioning principles; however, the primary difference between them is the type of USs used to support conditioning. In general, we talk about "fear conditioning" when aversive USs are used and we use the term "appetitive conditioning" when non-aversive USs are used.

Fear conditioning is expressed in most species. This cross-species feature has permitted animal data to provide important insights on human FC. In animals, the results observed may include the interruption of all locomotion and gross body movements during the presentation of the CS ("freezing"; see Bouton & Bolles, 1980), the suppression of ongoing instrumental behavior (i.e. conditioned emotional response; see Davis, 1990) and the amplification of the startle reflex (i.e. startle potentiation, Brown, Kalish, & Farber, 1951). In humans, some of the reactions typically seen when

confronted to CSs are autonomic nervous system responses (e.g. skin conductance response [SCR], fear potentiated startle [FPS], heart rate [HR] and blood pressure [BP]) and subjective measures of fear and anxiety through verbal report.

2.2 How do we study Fear Conditioning in humans?

Much of what we now today about fear learning is the result of research based in FC paradigms. These paradigms have shown to be an important instrument not only for studying the psychological processes underlying ADs but also for exploring the neurobiology of emotion and learning (Beckers et al., 2013) and the pathogenesis of ADs in the real world (Barlow, 2002). The usefulness of these procedures relies on the fact that is relatively easy to train rodents in FC paradigms and the fact that they can be easily implemented in humans.

2.2.1 Experimental Paradigms

The majority of FC paradigms consist of at least three phases: *habituation* (often called preconditioning), *conditioning* and *extinction.* Differential conditioning studies usually involve several initial exposures to both the CS+ and the CS- in random order and without any pairing. This is the habituation phase and its goal is to reduce orienting responses and to establish a physiological baseline. During the conditioning phase, only the CS+ is followed by the US. The extinction phase is often identical to the habituation

phase; however, in some cases the non-reinforcement rate is reduced from 100% to 50% to "slow" the extinction process.

Within FC paradigms, there are two major procedures used to assess the conditioned response, simple conditioning and differential conditioning. During simple conditioning, a single CS is repeatedly paired with the US. Simple conditioning is not influenced by inhibitory effects, only excitatory conditioning to the CS is measured. Within-subject conditioning effects are indexed by subtracting baseline or inter-trial-interval (ITI) levels of arousal from levels of arousal elicited by the CS. On the other hand, in a differential conditioning paradigm, there are two similar, initially neutral stimuli, one which is paired with the aversive US (CS+) and the other (CS-), which is presented without pairing. Here, differential learning is indexed as the difference between CR to the CS+ and CS- (Lissek et al., 2005a). Using this paradigm, an elevated response to the CS- in anxiety patients has been shown, suggesting an impaired differential learning (also called impaired fear inhibition) in PD (Lissek, 2009) and PTSD (Michael, Blechert, Vriends, Margraf, & Wilhelm, 2007; Jovanovic et al., 2010 respectively). Lissek's et al. (2005a) meta-analysis showed that patient-control differences in acquisition and extinction of simple conditioning were significantly larger than such differences in acquisition and extinction in differential conditioning paradigms. This patient-control difference in acquisition and extinction of simple conditioning implies greater activation of fear in the presence of danger cues (CS+) among patients, whereas the significantly smaller patient-control differences in

acquisition and extinction of differential learning may imply an impaired ability to suppress the fear response in the presence of safety cues (CS-) among anxiety patients.

A great variety of CSs (e.g. tone, figures, lights, faces) and USs (e.g. electric shocks, odors) have been used in FC studies. Lissek et al. (2005a) showed that CRs are higher when human faces are used as CSs, as compared to figures. This finding supports the idea that evolutionary prepared CSs are more aversive than non-prepared CSs.

2.2.2 Measures of Fear Conditioning in humans

2.2.2.1 Psychophysiological measures

Since the 1960s, the SCR has been the most popular index of FC in humans and one of the most robust and well-studied physiological responses (Milad, Igoe, & Orr, 2010). Some other terms that have been used to refer to SCR are galvanic skin response, psychogalvanic reflex and electrodermal response. SCR is caused by sympathetic nervous system activation, and primarily reflects the amount of sweat in the sweat glands. It is normally measured by attaching two electrodes to the individual's fingers of the palm hands, which are known to be highly responsive to emotional arousal. When someone is emotionally aroused, a change in the skin conductance level can be calculated as a measure of the magnitude of the SCR to a given stimulus.

The FPS has emerged as an important psychophysiological correlate of emotional activation (Lissek et al., 2005b). It was first described in animals by Brown, Kalish, & Farber (1951) and then extensively investigated by Davis (Davis, 1990). It is defined as

the increase in startle amplitude to startle stimuli delivered during the CS compared to startle stimuli delivered in the absence of the CS when a person is in a state of anxiety, and is considered as an operational measure of fear. It is commonly measured from the eye-blink related activity using electromyography of the orbicularis oculi, the muscle that controls closing of the eyelids. Some of the advantages of the FPS are that it provides an objective measure of fear responses, generates a non-zero baseline, offers cross-species generalization and is measured by a well-defined neural system (Davis, 1990).

Another procedure used as an index of FC has been the HR. It can be measured by means of an electrocardiogram, which measures electrical impulses associated with the contraction of the myocardial muscles of the heart, placing electrodes on particular areas of the body (e.g. chest, arms, legs). The amount of heart beats between repeated R-wave components of the electrocardiogram can be converted to a measure of HR. Other few conditioning studies have used BP to measure FC. It is monitored using a sphygmomanometer. BP is however a more intrusive measure and has been less utilized.

2.2.2.2 Subjective measures

Information regarding fear and anxiety caused by a given stimulus can be obtained via self-report through a variety of methods such as structured or unstructured interviews, questionnaires, schedules and inventories (Thyer, Papsdorf, Davis, &

Vallecorsa, 1984).

Ideally, subjective and physiological measures should produce synchronous results. However, it has been frequently demonstrated that the relationship between self-report measures of fear and the corresponding physiological indices are often in disagreement (see Schneider et al., 1999; Hermann, Ziegler, Birbaumer, & Flor, 2002; Veit et al., 2002; Lissek et al., 2008), although this varies according to the specific measures employed.

2.2.2.3 Neuroimaging measures

Neuroimaging studies represent a novel technique for investigating the neurobiological correlates of FC processes. Through functional magnetic resonance imaging (fMRI), neural substrates involved in FC can be assessed by measuring the blood-oxygen-level-dependant (BOLD) response. Currently, there is evidence that certain subnuclei within the amygdaloid complex differentially contribute to associative learning. A recent systematic review (Sehlmeyer et al., 2009) of neuroimaging studies on human FC and extinction showed the activation of a relatively common core fear network in the majority of studies, although there were important discrepancies. These inconsistencies are not surprising given the large methodological variety in imaging and design parameters as conditioning protocol (delay, trace), reinforcement rate (100%, 80%, 50% or less), contingency awareness, the modality of CSs and USs (tactile, auditory, visual, olfactory), and the concurrent assessment of the CR by other methods

(e.g. psychophysiological measurements or verbal ratings) (Sehlmeyer et al., 2009).

2.3 Role of Fear Conditioning in Anxiety Disorders

As stated above, FC studies in ADs implicate enhanced anxious reactivity to CSs that signal safety as an important correlate of clinical anxiety. It has been observed that anxiety patients fail to inhibit fear in the presence of safety cues, displaying fear responses to both CS+ and CS- (Orr et al, 2000; Peri, Ben Shakhar, Orr, & Shalev, 2000; Lissek et al., 2005a). This leads to low levels of differential learning even when patients actually do show conditioning to the CS+. Conversely, HC can suppress these fear responses during CS presentations showing higher rates of differential learning. This impaired discrimination between CS+ and CS- among patients may also be conceptualized as stimulus generalization. Both, stimulus generalization or impaired fear inhibition support the above predictions regarding differential learning (Lissek et al., 2005a).

An opposing theory for differential impairment across patients and controls is that of enhanced conditionability that predicts heightened differential conditioning among anxiety patients. Orr and colleagues (2000) asserted that individuals that are more likely to suffer from pathological anxiety might be more "conditionable" than healthy individuals (Orr et al., 2000; Peri et al., 2000). Here, conditionability can be defined as the extent to which responding to the CS+ exceeds CS-.

According to Lissek et al. (2005a), patient-control differences in acquisition of

simple conditioning implies a greater conditioned fear response in front of danger cues (CS+) among anxiety patients, whereas the significantly smaller patient-control differences in acquisition of differential learning may imply an impaired ability to suppress the fear response in the presence of safety cues (CS-). Many studies have evidenced this impairment in differential conditioning (Grillon & Morgan, 1998; Orr et al., 2000; Peri et al., 2000), suggesting a combination of stronger excitatory and weaker inhibitory associations in anxiety patients. These results demonstrate that associative learning occurrs in both groups, but only patients are unable to inhibit fear responses to the CS-. These findings parallel the theory of pathological anxiety (Davis, Falls, & Gewirtz, 2000) that implicates abnormalities in inhibitory fear mechanisms among anxiety patients. To sum up, research so far has well established that abnormalities in FC may underlie ADs.

Fear Conditioning in Social Anxiety Disorder

3. Fear Conditioning in Social Anxiety Disorder

3. 1 Social Anxiety Disorder: a prevalent and costly disorder

Social anxiety disorder is a common and debilitating anxiety disorder characterized by a persistent irrational fear of social/performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others, avoiding such situations or enduring them with marked distress (APA, 2000). Individuals with a SAD diagnosis frequently present comorbid mood, anxiety and substance abuse disorders, which are often secondary to SAD (Katzelnick & Greist, 2001). The disorder is often present since early adolescence and it is chronic in nature, and therefore it can seriously affect the educational, social, occupational and physical functioning of an individual, reducing his/her quality of life (Albano & Detweiler, 2001; Katzelnick & Greist, 2001). Although underrecognized and undertreated, SAD imposes high social and economical costs because of the expenses associated with treatment, reduced productivity, and absenteeism from work, among others (DuPont et al., 1996; Lépine, 2002).

3.1.1 Prevalence, comorbidity and gender differences

The first epidemiologic study assessing SAD, the Epidemiologic Catchment Area (ECA) found a lifetime prevalence of 2.8% (Schneier, Johnson, Hornig, Liebowitz, & Weissman, 1992) in the USA. Later, the National Comorbidity Survey (Magee, Eaton, Wittchen, McGonagle, & Kessler, 1996), also in the USA, found higher rates for SAD with

a lifetime prevalence of 13.3%. Weiller, Bisserbe, Boyer, Lepine, & Lecrubier (1996), as part of a WHO study in general health care in France, found a lifetime prevalence of 14.4%. Finally, the National Comorbidity Survey Replication found a lifetime prevalence of 12.1% for SAD (Kessler et al., 2005). In Europe, the ESEMeD project (Alonso et al., 2004) found a lifetime prevalence of 2.4%, although the corresponding figure was much lower in Spain: 0.6% (Haro et al., 2006). Although there is some variability regarding prevalence rates, most studies conclude that SAD is one of the most common mental disorders.

As mentioned above, comorbidity rates among SAD patients are very high. Approximately, 70-80% of individuals with SAD meet criteria for additional diagnoses, including specific phobia, agoraphobia, major depression and alcohol abuse and dependence (Magee et al., 1996). In general, it has been found that SAD most often precedes comorbid disorders (Schneier et al., 1992; Magee et al., 1996).

With regard to gender, some studies have suggested that, among ADs, gender differences are the least pronounced for SAD (Breslau, Chilcoat, Peterson & Schultz, 2000), with the average gender ratio ranging between 1:1 (Moutier & Stein, 1999) and 3:2 (Kessler et al., 2005). Both men and women with SAD appear equally likely to seek treatment (Katzelnick & Greist, 2001).

3.1.2 SAD subtypes

Much controversy exists regarding the nature of the difference between generalized and non-generalized subtypes of SAD. Social anxiety disorder was initially

conceptualized as a form of specific phobia. Then, the DSM-III (APA, 1980) stated that social and simple phobias often involve a circumscribed stimulus and that when more than one type is present, multiple diagnoses should be made. However, it still did not recognize the generalized and non-generalized subtypes of SAD stating that generally, individuals present only one social phobia. Later, in the DSM-III-R (APA, 1987), the generalized subtype diagnostic specifier was finally included if the person feared "most or all" social situations.

Nowadays, it is assumed that most individuals with SAD fear and avoid diverse social situations. The most commonly feared social situation is public speaking (Pollard & Henderson, 1988); others include initiating or maintaining conversations, going to parties, and dating and meeting strangers (Stemberger, Turner, Beidel & Calhoun, 1995). Feared performance situations include eating, writing, or urinating in public. Individuals who fear most of these situations are categorized as having generalized SAD, while individuals who do not fulfill this criterion are classified in the non-generalized subtype. Regarding this differentiation, it has been shown that generalized SAD is more associated with fear of interpersonal situations, whereas the non-generalized subtype is more associated with fear of performance situations (Stemberger et al., 1995; Cox, Clara, Sareen, & Stein, 2008).

It is common that researchers quantify the number and type of feared social situations on the basis of items reported in social anxiety questionnaires and other self-report instruments. Some studies have reported that individuals with generalized SAD score higher on self-report measures of social anxiety (Stein & Chavira, 1998), are also

more likely to have other comorbid Axis I diagnoses and more overall psychopathology, such as general anxiety, depression (Herbert, Hope, & Bellack, 1992; Holt, Heimberg, & Hope 1992; Hofmann & Roth, 1996) and neuroticism (Stemberger et al., 1995) than the non-generalized subtype. However, the fact that the generalized subtype of SAD is defined exclusively on the basis of the number and type of feared social situations, has contributed to many inconsistencies in distinguishing the subtypes. Another criticism regarding this issue is that the number of feared social situations is not an adequate method to identify and classify meaningful and qualitatively distinct subgroups (Stein, Torgrud, & Walker, 2000; Hofmann, Heinrichs & Moscovitch, 2004).

Despite the many attempts in clarifying and distinguishing between generalized and non-generalized SAD, there is still little evidence to support the subtyping of SAD (see Stein et al., 2010 or Aderka, Nickerson & Hoffman, 2012).

3.1.3 Origins of Social Anxiety Disorder

3.1.3.1 Familial and genetic factors

There is considerable consensus that the etiology of SAD is still far from being understood. There are several possible pathways and risk factors that may lead to the development and maintenance of SAD. Nowadays, there is enough evidence suggesting that familial factors play a modest but significant role in the development of the disorder. SAD is common among relatives of SAD individuals (Fyer, 1993; Fyer, Mannuzza, Chapman, Martin, & Klein, 1995). Other studies have concluded that children with SAD are more likely than their non-affected peers to have parents with SAD and

similarly the children of parents with SAD are at elevated risk for the disorder (Lieb et al., 2000). Moreover, some studies have reported a significant genetic component to social anxiety. For example, a meta-analysis of twin studies reported a heritability estimate for social anxiety of 0.65 (Beatty, Heisel, Hall, Levine, & La France, 2002), however other authors have reported more modest estimates of around 0.4–0.5 (Albano & Detweiler, 2001; Ollendick & Hirshfeld-Becker, 2002). It is noteworthy that, despite a common genetic factor shared across many ADs, some evidence suggests that certain genetic components are unique to social anxiety (Rapee & Spence, 2004). In a recent twin study, Sundet, Skre, Okkenhaug, & Tambs (2003) found that, in addition to genetic and environmental influences common to four fear dimensions (situational fears, illness–injury fears, social fears, and fear of small animals), there were significant fear-specific genetic and environmental factors. In the case of socials fears, genetic factors common across all fear types accounted only for 5% of the variance in social fears.

3.1.3.2 Temperamental factors

Other etiological models of SAD focus on early temperament styles. Currently, there is a good deal of evidence to suggest that children who manifest a behaviorally inhibited temperament style are at increased risk for the later development of SAD (Rapee & Spence, 2004; Mineka & Oehlberg, 2008). The construct of behavioral inhibition describes a relatively consistent pattern of behavioral and emotional responses to unfamiliar or novel people, places/situations, or objects. Inhibited children

typically respond to novel situations with initial restraint, caution, low rates of approach, and quiet withdrawal, and with unfamiliar people they are usually shy, timid, and reticent (Kagan, Reznick, Clarke, Snidman, & Garcia Coll, 1984). At least two studies have identified high rates of SAD among individuals who were identified as being highly behaviorally inhibited earlier in childhood (Biederman et al., 2001; Neal, Edelmann, & Glachan, 2002).

3.1.3.3 Cognitive and attentional factors

Cognitive and attentional processes may as well play a role in the origins and maintenance of pathological social anxiety. SAD is characterized by biases and distortions in social-information processing and thoughts, attitudes and beliefs that may trigger and maintain social phobic behaviors (Clark & Wells, 1995; Rapee & Heimberg, 1997). These models propose that SAD is maintained through a vicious cycle in which SAD individuals engage in a series of biased pre- and post-interaction cognitive processes that act to generate anxious emotion and may impair social performance. Subsequent social avoidance and adverse social outcomes are then proposed to reduce opportunity for further psychosocial development and perpetuate the assumption that social events will lead to negative outcomes (Clark & Wells, 1995; Rapee & Heimberg, 1997).

3.1.3.4 Social learning factors

A form of social learning that influences the expression of social anxiety is

vicarious conditioning. By observing the reactions of others to an environmental stimulus, and individual can learn to fear this stimulus (e.g. seeing another person behaving anxiously in a social situation). The strongest evidence for this pathway to the acquisition of fear comes from the primate model of phobic fear acquisition developed by Mineka and colleagues in the 1980s. These authors showed that naïve laboratory-reared rhesus monkeys that were not initially afraid of snakes rapidly acquired a phobic fear of snakes after watching a wild-reared model monkey behave fearfully in front of a snake (Mineka & Cook, 1986; 1993). Human psychophysiological studies, using electrodermal responses have also demonstrated vicarious acquisition of FC (Green & Osborne, 1985).

Social learning theories also suggest that a history of aversive outcomes from social interactions and lack of modeling or instruction regarding adaptive cognitive and behavioral coping strategies for managing challenging social situations may contribute to the development of social anxiety (Rapee & Spence, 2004). There is evidence of modeling of social anxiety in families of SAD patients. In families with one parent with SAD, offspring were more likely to present avoidant social behavior than offspring in families where parents had no SAD (Mineka & Zinbarg, 2006).

Finally, cultural norms may be also involved in the expression of social anxiety. The underlying continuum of social anxiety is likely to be constant across cultures, but the way in which the features are expressed as well as their consequences may differ depending on a given culture. The most know illustration is the Japanese disorder *taijin kyofusho*, the Western equivalent to the diagnosis of SAD. People with this disorder fear

that they will do something that can embarrass others, for example, by blushing (Mineka & Zinbarg, 2006). On the other hand, culture is likely to influence the point at which social anxiety is seen as an impediment in one's life. This may influence the rates at which high levels of social anxiety are diagnosed as a clinical condition in a particular cultural group. Given that most individuals spend all of their lives in a given culture (or subculture), these cultural pressures are likely to be of relevance to the expression of SAD across most of the lifespan (Rapee & Spence, 2004).

3.1.3.5 Neurobiological variables

Several hypotheses on the neurobiology of SAD exist. Among the many neurotransmitter systems involved in SAD, abnormalities in noradrenergic, serotonergic and dopaminergic systems seem to be the most relevant. For example, Bell, Malizia, & Nutt (1999), revealed noradrenergic abnormalities in both, the sympathetic nervous system and the central nervous system of individuals with SAD. Another study found altered serotoninergic activity in SAD (Potts, Davidson, Krishnan, Doraiswamy, & Ritchie, 1991). Furthermore, the therapeutic response to selective serotonin reuptake inhibitors (SSRIs) seems to support the role of serotonergic dysfunction in SAD (Van der Linden, Stein, & Van Balkom, 2000).

Studies in patients with SAD have also suggested a dysregulation of the dopaminergic system. Two neuroimaging studies in patients with SAD have shown changes in the striatal dopaminergic system. Tiihonen et al. (1997) found that SAD is associated with a reduction in D_2 receptor binding. Schneier et al. (2000) found that SAD

patients have decreased dopamine D_2 receptor binding potential compared to HC suggesting low dopamine system activity specific to this disorder.

Finally, as regards to neuroimaging, studies to date have suggested that paralimbic, striatal and neocortical regions play a role in SAD (Mathew, Coplan, & Gorman, 2001; Marcin & Nemeroff, 2003). In any case, although neuroimaging studies represent a potential instrument to a better understanding of this disorder, the neurobiology underlying SAD is still poorly understood.

3.1.3.6 Fear conditioning theories

Finally, FC theories provide another plausible explanation for the onset and/or maintenance of social fears. Early conditioning models assumed that traumatic conditioning experiences were necessary for the development of phobic fears. For example, Stemberger et al. (1995) found that 56% of those with non-generalized SAD recalled direct traumatic conditioning experiences as having played a role in the origins of their SAD. These aversive social experiences often include excessive teasing, criticism, bullying, rejection, ridicule, humiliation, or exclusion by significant others (Rapee & Spence, 2004). The specific role of FC in the development of SAD will be discussed in detail in the next section.

3. 2 Role of Fear Conditioning in Social Anxiety Disorder

According to Rachman (1977), there are at least three major pathways to

acquiring fear. He proposed that fears could be acquired through aversive classical conditioning after a traumatic event, vicariously and by transmission of information and/or instruction. Informational and instructional processes provide the basis for most of our commonly encountered fears of everyday life. We do not only learn which situation to fear, but we also learn to distinguish those situations and objects that are not dangerous, and therefore, not fear them. This non-random quality of fears is best explained by the preparedness theory proposed by Seligman. In this view, it is assumed that evolutionary pressures have predisposed primates to condition fear more readily to stimuli related to recurrent survival threats (phylogenetically fear-relevant stimuli) than to stimuli that never have threatened survival (fear-irrelevant stimuli) (Seligman, 1971).

In this regards, Öhman & Mineka (2001), have argued that SAD may be regarded as a consequence to associate threatening social stimuli such as angry faces with an aversive event. These conditioning processes are thought to contribute to the development of SAD by conferring anxiogenic valence to the CSs (and stimuli resembling the CS), that are then capable of eliciting and maintaining anxiety responses in the absence of an actual CS-US contingency. For example, a highly humiliating public speaking experience (US) could be associated with certain people or places (CS) and contribute to the persistence of social anxiety beyond the presence of USs in SAD individuals.

3.2.1 Non-experimental evidence

McCabe, Antony, Summerfeldt, Liss, & Swinson (2003) found a significant

relationship between self-reported history of teasing and 26 individuals with SAD. When participants were asked a series of questions about their prior life experiences, 92% of the sample reported severe teasing and bullying experiences.

Hackmann, Clarke, & McManus (2000) assessed 22 patients with SAD with a semi-structured interview that explored the nature of social phobic imagery. They found that the entire sample of participants with SAD reported experiencing recurrent negative images in social situations, with 96% of the sample reporting that the image was associated with a particular negative memory and 77% reporting that the event increased their anxiety in social situations. The authors speculated that, in individuals with SAD, early unpleasant social experiences (e.g. being bullied by peers) might lead to the development of a negative, observer-perspective image of the social self that is activated in subsequent anxiety-provoking situations.

Another study that assessed 68 individuals with specific or generalized SAD found that 56% of those with specific social phobias had memories of direct traumatic conditioning experiences linking them with the onset of their social anxiety (Stemberger et al., 1995).

Memories of childhood teasing in a non-clinical sample have also been related with increased levels of social anxiety (Roth, Coles, & Heimberg, 2002). Such findings raise the possibility that memories of childhood teasing may be a non-specific risk factor for a range of anxiety conditions. Taken together, these findings suggest that conditioning experiences may play a role in the development of SAD.

3.2.2 Experimental studies using socially-irrelevant stimuli

Schneider et al. (1999) used a differential aversive conditioning paradigm in 12 generalized SAD individuals and 12 HC, using neutral faces as CS and an unpleasant odor as US. Heart rate and subjective ratings were used to measure FC. Results indicated that there was evidence of conditioning in subjective measures in both groups but there were no between-group differences in conditioning as measured by HR. Using similar procedures, Hermann et al. (2002) assessed the role of FC in 14 SAD individuals and 19 HC predicting a more intense CR at the subjective and physiological level in SAD compared to HC. Both groups showed CR as reflected by subjective measures, SCR and FPS. Nevertheless there was no evidence for an enhanced FC among SAD subjects relative to HC. Another FC study by Veit et al. (2002) using neutral faces as CS and painful pressure as US compared 4 SAD individuals and 7 HC. The authors found an enhanced SCR to the CS+ in HC but not in SAD individuals. A CR as measured by subjective ratings was also found, but again there were no group differences.

In sum, all three studies using socially irrelevant USs, failed to show enhanced conditionability in SAD patients compared to HC, whether the CR was skin conductance, FPS or HR (See Table 2).

3.2.3 Importance of using disorder-specific US: the specificity hypothesis

Until recently, most human studies using FC paradigms have used fairly simple

procedures consisting of pairing irrelevant USs, such as light or geometric figures, with the presence and/or absence of an electrical shock or other aversive stimuli such as an unpleasant odor. As stated before, results using these paradigms have not shown that SAD is characterized by enhanced FC (Schneider et al., 1999, Hermann et al., 2002, Veit et al., 2002).

Of specific importance are studies using socially relevant USs (e.g. insults) because these resemble more closely the socially aversive events related to the etiology of the disorder (Pejic, Hermann, Vaitl, & Stark, 2011). A recently developed, ecologically-enhanced social conditioning paradigm with socially relevant stimuli as the US showed enhanced FC in SAD patients. In this study, Lissek et al. (2008) compared 20 individuals with SAD and 18 HC in a differential FC paradigm using negative insults with critical faces as USs and neutral faces as CSs. Only individuals with SAD displayed evidence of FC to the CSs. Additionally, two recent neuroimaging studies in healthy subjects using socially relevant USs found that social anxiety was associated with enhanced FC. Davis, Johnstone, Mazzulla, Oler, & Whalen (2010) assessed regional differences in amygdala response to explore the neural correlates of social conditioning in 47 non-clinical participants using neutral facial expressions as CSs and self-relevant sentences as USs. Results showed an increased BOLD response in the lateral ventral region of the amygdala during faces predicting negative social outcomes, suggesting an enhanced acquisition of CRs. Pejic et al. (2011), investigated the neural basis of fear acquisition in 49 non-clinical participants within a 2-day fMRI differential conditioning protocol. They found an enhanced activation in the left amygdala and trends in the right dorsal anterior

cingulate cortex and the left hippocampus while observing neutral facial expressions (CSs) paired with film-clips of critical comments (USs) demonstrating that social anxiety is related to altered social threat learning. Overall, both studies suggest a relationship between social anxiety and conditionability to socially threatening stimuli.

These results evidenced that the qualitative nature of the CR depends on the qualitative nature of the CSs. This is consistent with the idea that the closer and more similar the CSs are to the USs, the stronger the CR will be (see Bouton, Mineka & Barlow, 2001). Applied to the clinical world, this "specificity hypothesis" implies that using socially relevant stimuli as the US should elicit "higher conditioning" in SAD compared to other anxiety patients (as interoceptive cues should elicit "higher conditioning" in PD versus other ADs; see Acheson, Forsyth, Prenoveau, & Bouton, 2007; De Cort, Griez, Büchler, & Schruers). However, determining whether this enhanced conditionability to socially relevant USs is specific to SAD in comparison to other ADs remains to be tested. Table 2 summarizes all studies in FC using socially relevant stimuli.

Table 2. Studies of Fear Conditioning in Social Anxiety

Study	*N* SAD (HC)	CS	US	DV	Results
Clinical Samples					
Schneider et al. (1999)	12 (12)	Neutral faces	Odor	fMRI	Enhanced amygdala and hippocampus activation in SAD vs HC
					FC in SAD > FC in HC
				HR	No FC in SAD, No FC in HC
Veit et al. (2002)	4 (7)	Neutral faces	Painful pressure	fMRI	Enhanced amygdala, OFC, insulae, ACC and DLPFC activation in SAD vs HC
					FC in SAD > FC in HC
				SCR	FC in HC, No FC in SAD
Hermann et al. (2002)	14 (19)	Neutral faces	Odor	FPS	FC in SAD = FC in HC
				SCR	FC in SAD = FC in HC
				HR	No FC in SAD, No FC in HC
Lissek et al. (2008)	20 (18)	Neutral faces	Negative insults + critical faces	FPS	FC in SAD > FC in HC
Non-clinical Samples					
Davis et al. (2010)	(42)	Neutral faces	Insults	fMRI	Enhanced amygdala activation during neutral faces paired with insults (CS+) vs neutral faces paired with compliments (CS-).
Pejic et al. (2011)	(49)	Neutral faces	Critical comments	fMRI	Enhanced amygdala activation during neutral faces paired with critical comments (CS+) vs neutral faces alone.
				SCR	No evidence of FC to the CS+.

CS, Conditioned Stimuli; US, Unconditioned Stimuli; DV, Dependent Variable; fMRI, functional magnetic resonance imaging; HR, Heart Rate; SCR, Skin Conductance Response; FPS, Fear Potentiated Startle; CR, Conditioned Response; ACC, Anterior Cingulate Cortex; SAD, Social Anxiety Disorder; HC, Healthy Controls.

3. 3 Role of Fear Extinction in Social Anxiety Disorder

In addition to abnormalities in fear acquisition, failure to extinguish conditioned fear may also be important in the pathogenesis of SAD (Davis, Falls, & Gewirtz, 2000; Milad, Rauch, Pitman, & Quirk, 2006). According to Mineka & Zinbarg (1996), delayed fear extinction could account for the observed persistence of social fears.

To date, only two studies using socially relevant USs have investigated the role of fear extinction in SAD and their findings were in the opposite direction to the predictions of Mineka & Zinbarg (1996). Lissek et al. (2008) and Pejic et al. (2011) found no delayed fear extinction in SAD individuals compared to HC, neither for subjective data nor for psychophysiological measures. However, in the study by Pejic et al (2011), BOLD responses assessed with fMRI data did reflect a negative correlation between levels of social anxiety and neural activation, suggesting a delayed extinction in those with higher levels of social anxiety.

The role of fear extinction in SAD and ADs in general is of enormous importance because extinction learning underlies the treatment technique known as exposure therapy. Exposure therapy consists of a systematic exposure to the feared CS in the absence of any negative reinforcement. After several sessions of exposure therapy, the majority of patients learn to inhibit their fear responses. Understanding the mechanisms of fear extinction may help elucidate the pathophysiology of ADs and the mechanism of action underlying extinction-based therapies.

3.4 Clinical relevance

Fear conditioning models have been considered a prime tool for the experimental study of psychopathology and have provided a laboratory model for the pathogenesis of ADs in the "real world" (Mineka & Zinbarg, 2006). Fear conditioning models can be advantageous given that they provoke the typical behavioral symptoms of ADs (e.g. avoidance) and can be used to test ways of reducing these symptoms by means of extinction processes. The relationship between conditioning models and ADs continues to be of great value for the development of new techniques that may reduce pathological anxiety and to counteract relapse after successful treatment (Culver, Stoyanova, & Craske, 2011).

On the other hand, fear extinction models have been used to study the underlying mechanisms of dysfunction across ADs and examine potential biomarkers. A key element of ADs is the inability to inhibit or extinguish fear (Otto, Smits, & Reese, 2004). Fear extinction models offer a direct measure for this specific impairment. Delayed or impaired extinction might be a precursor of anxiety and its early detection may be useful to predict vulnerability for ADs.

As mentioned above, fear extinction models can also be used as a pre-clinical model of exposure therapy. Since exposure therapy is an analog of extinction learning, laboratory-based research on fear extinction has been successful in providing ways that can augment the efficacy of exposure therapy (Milad et al., 2006; Graham & Milad, 2011). One promising contribution has been the use of D-cycloserine, an agonist at the

glutamatergic N-methyl-D-aspartate receptor to enhance exposure therapy outcome in several ADs, including SAD (Hofmann et al, 2006). Several studies suggest that methods that facilitate fear extinction are an important avenue in the treatment of fear disorders (Graham & Milad, 2011).

Finally, extinction research has been mainly based on work with laboratory rodents and many of its findings are being generalized to humans. The cross-species validity of extinction models permits animal research to be compared with human studies, because of considerable similarity in the neural structures involved.

EXPERIMENTAL APPROACH

4. Experimental Approach

4.1 General Approach

The overall goal of the present dissertation was to investigate FC processes in social anxiety. We wanted to test the hypotheses that abnormal FC characterizes social anxiety and that abnormal FC to socially relevant USs is specific of SAD compared to other ADs.

To address this goal, we conducted two separate studies using a novel paradigm developed by Lissek et al. (2008). In Study 1, we compared social conditioning across two clinical groups: SAD and PDA, and HC. To our knowledge, this would be the first attempt to replicate the Lissek et al. study (differences between SAD patients and HC) and the first test of the "specificity hypothesis" (differences between SAD and PDA patients). In Study 2, we tested whether enhanced FC was also evident in individuals "at-risk" for SAD. To this end, we compared social conditioning across individuals with sub-clinical social anxiety (SSA) and HC.

4.2 Objectives

1. To adapt to the Spanish cultural context a paradigm for the study of fear conditioning based on socially relevant USs.
2. To assess fear conditioning in SAD and SSA within a novel conditioning paradigm using socially relevant USs compared to other anxiety disorders (PDA) and HC.
3. To study the relationship between the degree of fear conditioning and social anxiety.

4.3 Hypotheses

1. The paradigm for the study of fear conditioning based on socially relevant USs will be successfully adapted to the Spanish cultural context.

2. Patients with SAD and SSA will present enhanced fear conditioning to socially relevant US in comparison to PDA patients and HC.

3. There will be a significant positive correlation between the degree of fear conditioning to socially relevant USs and social anxiety in the clinical (SAD, PDA) and subclinical (SSA) sample and in HC.

METHOD

5. Method

5.1 Participants

Approximately 700 university students from two universities from Barcelona (Spain) were screened using the Spanish self-report version of the Liebowitz Social Anxiety Scale (LSAS-SR; Bobes, 1999) via a web system. On the basis of their LSAS-SR scores, 120 of these individuals were interviewed by a licensed clinical psychologist not involved in the experimental phase using the Spanish version of the Mini International Neuropsychiatric Interview (MINI, Sheehan et al., 1998). The MINI has shown to have good concordance with other diagnostic measures, and good interrater and test-retest reliability (Lecrubier et al., 1997; Sheehan et al., 1998). In a previous study in a Spanish sample, the sensitivity of SAD diagnosis was 100 and its specificity was 84,2 (Bobes, 1998).

Participants who fulfilled the inclusion/exclusion criteria and agreed to participate in the experimental phase (*n*=68) were allocated to one of the following groups: SAD *(n*=12, LSAS>50 and current SAD diagnosis) or HC (*n*=16, LSAS<20, and no current mental disorder) (Study 1) and SSA (*n*=20, LSAS>50 and no current mental disorder) or HC (*n*=20, LSAS<20, and no current mental disorder) (Study 2). The HC for each study were selected from the initial screening sample based on their LSAS score and optimal matching to the SAD/PDA groups or to the SSA group with regards to age and gender. Four additional SAD participants and all the participants in the PDA group *(n*=16, current PDA diagnosis) in Study 1 were recruited from the Anxiety Unit of the

Hospital del Mar (Barcelona, Spain) (see Figure 1). The same clinical psychologist using the MINI also assessed these participants.

Of the 16 participants with SAD, 12 met the criteria for generalized SAD and 4 met the criteria for non-generalized SAD[1]. The exclusion criteria for all participants included: 1) use of pharmacological medication (except for the PDA group, see below) or presence of medical pathology capable of interfering with the study objectives, 2) use of illicit drugs, 3) pregnancy and 4) not speaking Spanish. Additional exclusion criteria for the SAD and PDA subjects included: 1) current or past history of bipolar disorder, psychosis or delusional disorder, 2) current major depressive disorder, post-traumatic stress disorder or suicidal ideation, 3) alcohol or substance abuse or dependence (other than nicotine) during the last 3 months and 4) psychomotor delay. Participants in the PDA group could be taking antidepressants (SSRI) provided that they had been on a stable dose for at least 3 months prior to the study. All participants were Caucasian.

[1] We operationalized "generalized SAD" as having clinically significant anxiety (fear or avoidance score=3) in at least four different social situations, as assessed by the LSAS. The other participants were classified in the "nongeneralized SAD" group.

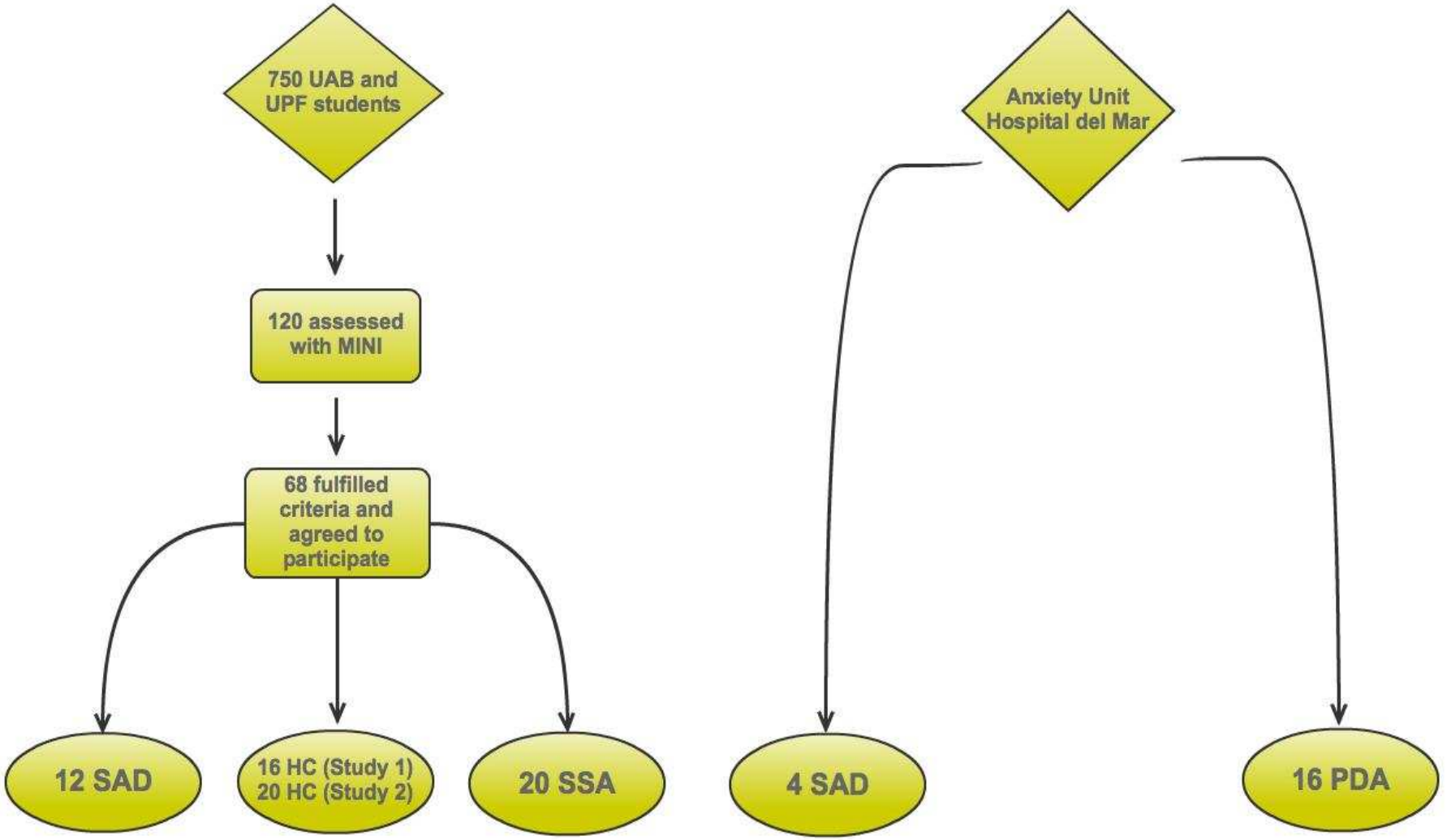

Figure 1. **Sample recruitment chart**

5. 2 Procedure

We used the paradigm developed by Lissek et al. (2008), with slight modification (adding a reminder phase and using a shorter extinction phase). The subjects participated in a differential FC procedure in which 8 s neutral facial expressions from three female actors (blonde, brunette, redhead) served as the CS and were paired with one of three types of a 3 s, 85 dB audiovisual US: 1) insults and critical facial expressions (US_{neg}); 2) comments and neutral facial expressions (US_{neu}); and 3) compliments and positive facial expressions (US_{pos}). The conditioned stimuli paired with the negative, neutral, and positive USs will be referred to as $CS_{neg,}$ $CS_{neu,}$ and $CS_{pos,}$ respectively.

The experiment consisted of four phases: *preconditioning* - 4 presentations of each type of CS in absence of the US; *conditioning* - 8 presentations of each type of CS immediately paired with its respective US; *reminder* - 4 presentations of each type of CS immediately paired with its respective US and *extinction* - 4 presentations of each type of CS in absence of the US. The preconditioning and conditioning phases were identical to those of Lissek et al. (2008). The reminder phase followed a 5 min break during which participants filled out the questionnaires. The reminder phase was added to ensure intact conditioning performance at the start of the extinction phase. The stimuli were presented in a quasi-random order, with the constraint of a maximum of two equal consecutive presentations. The assignment of faces to a type of CS was counterbalanced across subjects (see Figure 2).

	Conditioned Stimuli	Unconditioned Stimuli	
Positive			¡Me gustas! ¡Me alegro tanto de verte! ¡Estoy de acuerdo contigo! ¡Te veo muy bien hoy! ¡Estupendo! ¡Gracias por venir! ¡Eres genial! ¡Fantástico!
Neutral			Esta casa es grande. Necesito un bolígrafo. ¿Qué hora es? Acabo de volver. Necesito algún cambio. ¿Va llover? Necesito un lápiz. Hace calor.
Negative			¡No me gustas! ¡Vete! ¡Deja de mirarme! ¿Otra vez? ¡Basta ya! ¡Me das asco! ¿Qué pasa contigo? ¡Imbécil!

Figure 2. Schematic Summary of Lissek's Social Conditioning Paradigm translated into Spanish

During the experimental session all participants completed the LSAS-SR[2], the Spanish versions of the State Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1982), the Fear of Negative Evaluation Scale (FNES; García–López, Olivares, Hidalgo, Beidel, & Turner, 2001) and the Beck Depression Inventory (BDI; Sanz, García-Vera, Espinosa, Fortún, & Vázquez, 2005). In addition, the PDA patients completed the Spanish version (Bulbena & Martín-Santos, 1999) of the self-report version of the Panic Disorder Severity Scale (PDSS-SR; Houck, Spiegel, Shear & Rucci, 2002) (for questionnaires see Appendices section).

All the participants were paid 15€ and, after the experimental procedure was described in detail, were asked to sign an informed consent form, which was previously approved by the corresponding institution's Ethical Research Committee.

5.3 Physiological Recordings and Subjective Ratings

A BIOPAC Mod. MP150WSW psychophysiological recording system was used for stimulation and recording. The startle probe consisted of a 40 ms, 3.5 psi airpuff delivered through a polyethylene tube affixed 1 cm from the participants' foreheads. The startle blink reflex was recorded electromyographically (EMG) using two 5 mm silver surface electrodes placed under the left eye. Impedance levels were maintained below 5 KΩ. The EMG signals were amplified using a 10-500 Hz bandpass analog filter and were digitalized at a rate of 2000 Hz.

[2] Data presented in Tables 1 and 2 correspond to the second administration of the LSAS

The experimental phase began with 9 startle probes. During all four phases, the startle probes were delivered 4 or 5 s after CS onset. An 18-25 s ITI was maintained throughout the whole experiment. Ratings of anxiety, unpleasantness and arousal for the CS were obtained from participants on a 10-point Likert scale following conditioning and extinction. Additionally, between the conditioning and reminder phases, the participants rated each type of US by reflecting on the level of anxiety and happiness elicited using a 10-point Likert scale.

5. 4 Data Analysis

The startle EMG data were rectified and smoothed within a 20 ms moving window. The blink onset latency window was 20-100 ms and the peak magnitude was determined within 150 ms following response onset. The average EMG baseline levels for the 50 ms preceding the startle probe were subtracted from the peak magnitude. The EMG magnitudes were standardized using within subject T-score conversions to normalize the data. The conditioning phase was divided into 4 blocks, and the preconditioning, reminder and extinction phases were divided in 2 blocks each, one for each type of CS. For FPS measures, FC was operationalized as the difference between the CS_{neg} and the CS_{pos} during the conditioning and the reminder phase, and as the difference between the CS_{neg} and the CS_{pos} in early (first block) versus late (last block) during extinction.

In both studies, the EMG data were analyzed using analyses of variance (GLM procedure). In Study 1, we used a 3x2x3x4 design for the conditioning phases and a 3x2x3x2 design for the preconditioning, reminder and extinction phases, where subject group (SAD, PDA, HC) and gender were the between subject factors, and type of CS (negative, neutral, positive) and time (block 1, 2, 3 and 4 for conditioning; block 1 and 2 for preconditioning, reminder and extinction) were the within-subject factors. In Study 2, we used a 2x2x3x4 design for the conditioning phase and a 2x2x3x2 design for the preconditioning, reminder and extinction phases. Subject group (SSA, HC) and gender were the between-subject factors, and type of CS (negative, neutral, positive) and time (block 1, 2, 3 and 4 for conditioning; block 1 and 2 for preconditioning, reminder and extinction) were the within-subject factors. In both studies, simple contrasts were calculated to specify the main or interaction effects.

Self-report reactions to the CS (anxiety, unpleasantness and arousal) were also analyzed using a GLM procedure with a 3x2x3 (Study 1) or 2x2x3 (Study 2) design. Subject group and gender were the between-subject factors, and type of CS was the within-subject factor.

Eta squared values or Cohen's *d* were calculated as measures of effect size. Gender results are not presented given that the main and interaction effects of gender were not significant for either EMG or the self-report data in both experiments (all *F*s < 2.90, all *p*s > .11, all η_p^2 < .07). Also, BDI, STAIT-T and airpuff ratings were not found to interact with any of the dependent measures (all *F*s < 2.48, all *p*s > .10, all η_p^2 < .07).

Furthermore, to assess the relationship between severity of social anxiety and FC, LSAS and FNES scores were correlated with the CR during the conditioning phase using bivariate Pearson's correlations.

RESULTS

6. Results

6.1 Adaptation of the paradigm

The FC paradigm developed by Lissek et al. (2008) to assess FC correlates of SAD was adapted to the Spanish culture. The adaptation consisted in translating the insults, compliments and neutral comments (US) to Spanish and then, recording them. Recordings were done by the Faculty of Communication Studies of the Autonomous University of Barcelona. Translations are reported in Table 3.

Table 3. Translations of the US from English into Spanish

POSITIVE	You seem nice!	¡Me gustas!
	It is so good to see you!	¡Me alegro tanto de verte!
	I agree with you!	¡Estoy de acuerdo contigo!
	You're looking good today!	¡Te veo muy bien hoy!
	Terrific!	¡Estupendo!
	Thanks for coming down!	¡Gracias por venir!
	You are the best!	¡Eres genial!
	Fantastic!	¡Fantástico!
NEUTRAL	This is a big house.	Esta casa es grande.
	I need a pen.	Necesito un bolígrafo.
	What time is it?	¿Qué hora es?
	I just got back.	Acabo de volver.
	I need some change.	Necesito algún cambio.
	Is it going to rain?	¿Va llover?
	I need a footstool.	Necesito un lápiz.
	It is warm today.	Hace calor.
NEGATIVE	I don't like you!	¡No me gustas!
	Go away!	¡Vete!
	Stop looking at me!	¡Deja de mirarme!
	Again?	¿Otra vez?
	Stop already!	¡Basta ya!
	You disgust me!	¡Me das asco!
	What is wrong with you?	¿Qué pasa contigo?
	Stupid!	¡Imbécil!

Once the paradigm was translated, we conducted one pilot study in a small sample of healthy individuals (n=5) to assess conditioning degree and the subjective ratings to the US. Our results matched those of Lissek's with HC.

6.2 Fear Conditioning in SAD. Study 1.

The demographic and clinical characteristics of the participants in Study 1 are reported in Table 4.

6.2.1 Startle EMG

Preconditioning. The main effects of type of CS, type of CS by subject group and type of CS by time interaction were not significant (all *F*s < 1.75, all *p*s > .30, all η_p^2 < .08).

Conditioning. Although a significant type of CS by time interaction emerged for the whole sample ($F(6, 234) = 2.28$, $p = .04$, $\eta_p^2 = .06$), follow-up analyses showed that the responses to the CS_{neg} and CS_{pos} were not different in any block during conditioning (all *p*s > .54). Furthermore, the main effect of type of CS and the interactions type of CS by subject group and type of CS by time by subject group were not significant (all *F*s < 1.59, all *p*s > .19, all η_p^2 < .08; Figure 3).

Reminder. During the reminder phase, the main effect of type of CS and all the interactions with type of CS were not significant (all *F*s < 2.22, all *p*s > .12, all η_p^2 < .06).

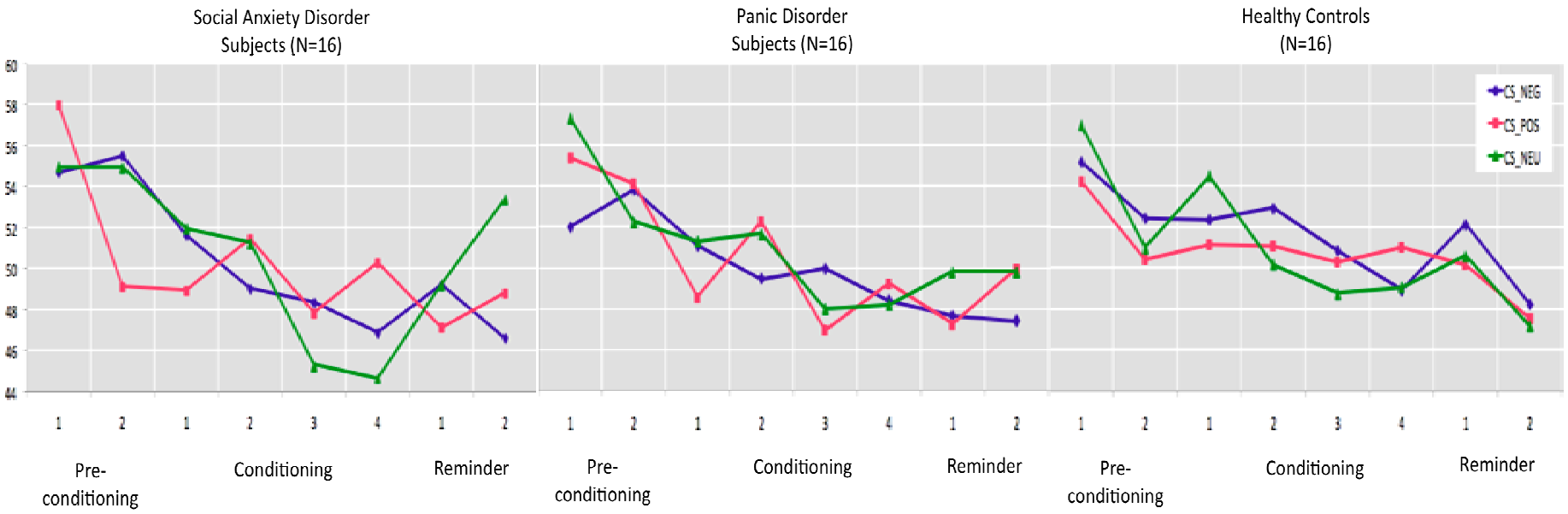

Figure 3. Average, Standardized Startle-Blink EMG Magnitudes During Preconditioning, Conditioning and Reminder phases

Extinction. During extinction, the main effects of type of CS and all the interactions with type of CS were not significant (all *F*s < .61, all *p*s > .60, all η_p^2 < .03).

6.2.2 Self-reported reactions to the conditioned stimulus

Preconditioning. The main effects of type of CS and type of CS by subject group interaction were not significant for all self-report measures (all Fs < .94, all *p*s > .40, all η_p^2 < .04).

Conditioning. We found evidence of conditioning at the subjective level for the whole sample, as revealed by a significant main effect of type of CS for the self-report measures of anxiety ($F(2, 84) = 8.82$, $p < .001$, $\eta_p^2 = .17$), unpleasantness ($F(2, 71.35) = 12.89$, $p < .001$, $\eta_p^2 = .24$) and arousal ($F(2, 84) = 15.29$, $p = < .001$, $\eta_p^2 = .27$). There were linear increases from CS_{pos} to CS_{neu} to CS_{neg} for anxiety ($F(1, 42) = 9.87$, $p = .003$, $\eta_p^2 = .19$) and unpleasantness ($F(1, 42) = 14.55$, $p < .001$, $\eta_p^2 = .26$) and there was a quadratic trend for arousal ($F(1, 42) = 28.50$, $p < .001$, $\eta_p^2 = .40$; see Figure 4). However, conditioning did not differ between groups, as indicated by the non-significant interaction between type of CS and subject group for any of these variables (all *F*s < 1.60, all *p*s > .18, all η_p^2 < .07). A posthoc test comparing the CS_{neg} vs CS_{pos} scores for each subjective variable (anxiety, unpleasantness, and arousal) confirmed the absence of differences in conditioning across groups (all *F*s < 1.19, all *p*s > .31, all η_p^2 < .05).

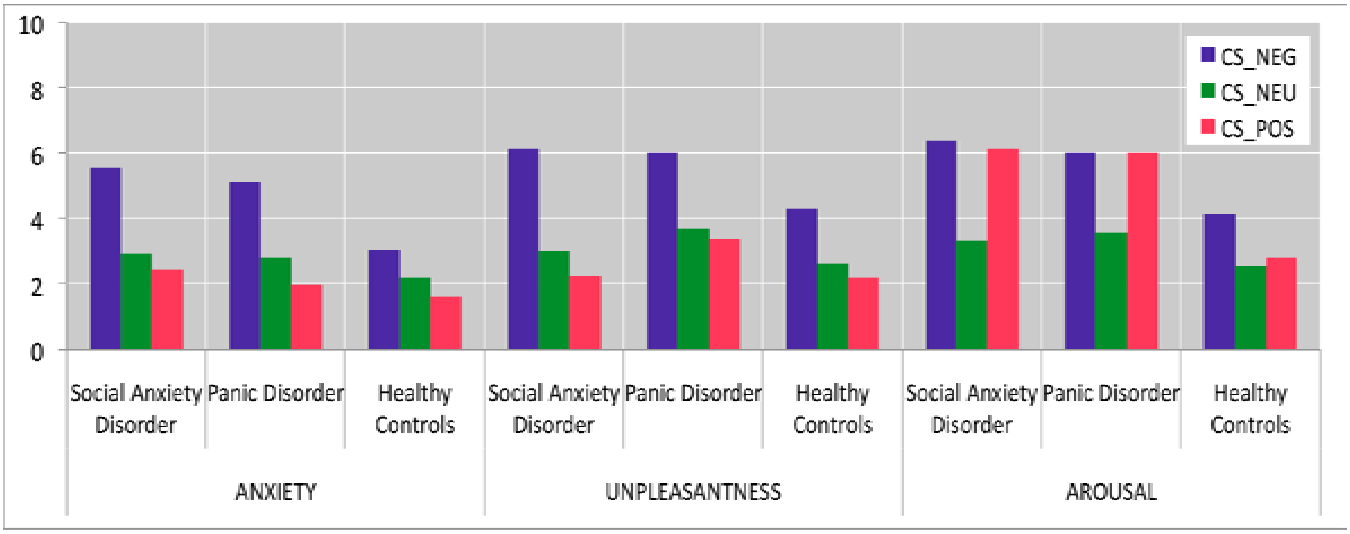

Figure 4. Likert Scale Scores for Self-Report Measures of Anxiety, Unpleasantness, and Arousal During Conditioning

Extinction. During extinction, and similar to conditioning, main effects of type of CS were found for anxiety, unpleasantness, and arousal (all *F*s > 9.31, all *p*s < .001, all η_p^2 > .18), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for anxiety ($F(1, 42) = 9.10$, $p = .004$, $\eta_p^2 = .18$) and unpleasantness ($F(1, 42) = 18.68$, $p < .001$, $\eta_p^2 > .31$), and a quadratic trend for arousal ($F(1, 42) = 24.97$, $p < .001$, $\eta_p^2 = .37$; see Figure 5). The type of CS by subject group interactions with anxiety and unpleasantness were not significant (both Fs < 1.65, ps > .17, ηp2 = .07) but significance was approached for the arousal ratings (F(4, 84) = 2.28, p = .07, ηp2 = .10). Pair-wise comparisons revealed that the arousal ratings for the CS_{pos} were significantly higher for SAD ($t(47) = 2.44$, $p = .05$, $d = .35$) and PDA patients ($t(47) = 2.61$, $p = .04$, $d = .37$) in comparison to HC.

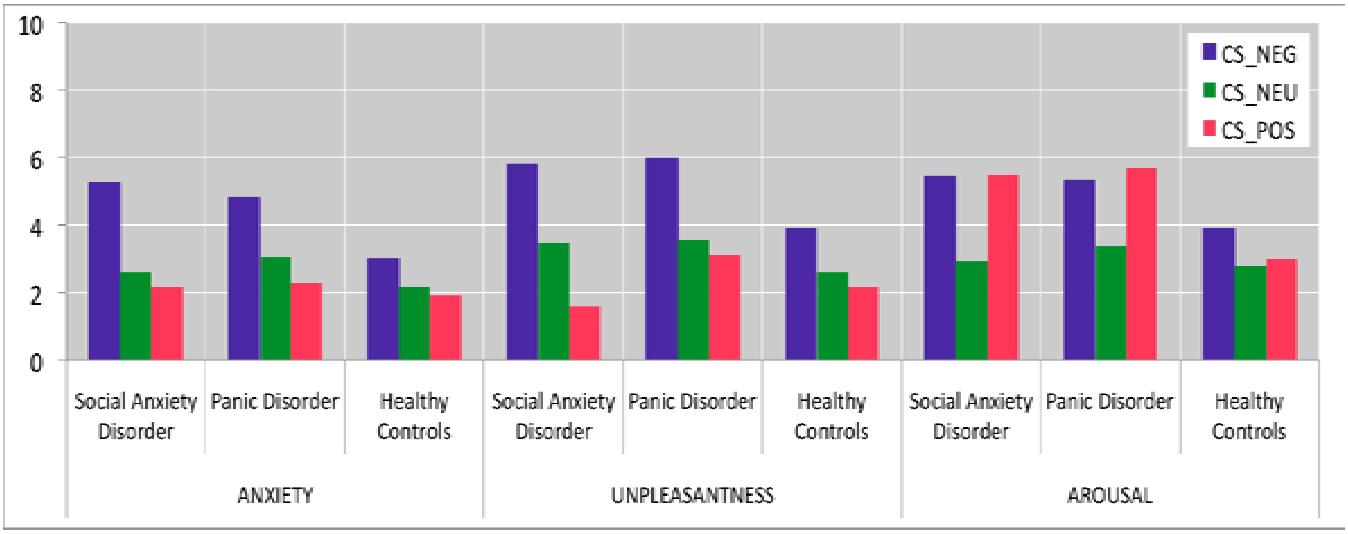

Figure 5. Likert Scale Scores for Self-Report Measures of Anxiety, Unpleasantness, and Arousal During Extinction

6.2.3 Self-report reactions to the unconditioned stimulus

Main effects of type of US were found for the self-report measures of happiness ($F(2, 63.09) = 50.67$, $p < .001$, $\eta_p^2 = .55$) and anxiety ($F(2, 73.35) = 20.53$, $p < .001$, $\eta_p^2 = .33$), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for the anxiety ratings ($F(1, 42) = 23.84$, $p < .001$, $\eta_p^2 = .36$) and linear decreases from CS_{pos} to CS_{neu} to CS_{neg} for the happiness ratings ($F(1, 42) > 66.79$, $p < .001$, $\eta_p^2 = .61$; see Figure 6). However, there were no differences in the happiness or anxiety ratings of the US between groups, as shown by the non-significant type of US by group interactions (both Fs < .89, both ps > .47, all $\eta_p^2 < .04$).

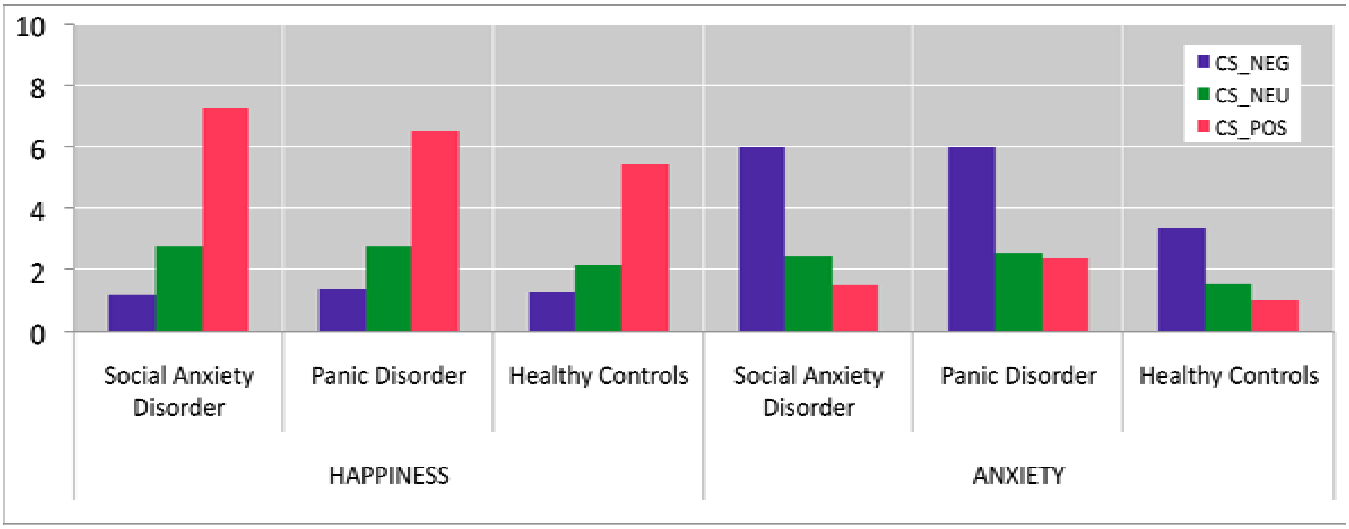

Figure 6. Likert Scale Scores for Self-Report Measures of Happiness and Anxiety to the US

6.2.4 Comorbidity, medication, and SAD subtype

We repeated the above analyses but excluded the three participants from the SAD group who had comorbid GAD (all *F*s < 3.90, all *p*s > .10, all η_p^2 < .09), the participants in the PDA group who were taking medication (all *F*s < 2.65, all *p*s > .10, all η_p^2 < .08) and the four SAD participants who did not meet the criteria for generalized SAD (all *F*s < 2.90, all *p*s > .11, all η_p^2 < .07). None of these analyses had any significant effect on the results.

6.3 Fear Conditioning in Sub-clinical Social Anxiety. Study 2.

The demographic and psychometric characteristics of the participants in Study 2 are reported in Table 5.

6.3.1 Startle EMG

Preconditioning. The main effects of type of CS, type of CS by subject group and type of CS by time interactions were not significant (all *F*s < 2.13, all *p*s > .13, all η_p^2 < .06).

Conditioning. Although a significant type of CS by time interaction emerged (*F*(6, 172.2) = 3.57, *p* < .005, η_p^2 = .10) and follow-up analyses revealed significant differences between the CS_{neg} and CS_{pos} (*t*(39) = 3.56, *p* < .005, *d* = .56) for the whole sample, these interactions were in the opposite direction than we predicted, with higher responses to the CS_{pos} than to the CS_{neg} in the fourth block of the conditioning phase. In any case, the main effects of type of CS and the interactions between type of CS and subject group and between type of CS by time and subject group were not significant (all *F*s < 1.58, all *p*s > .15, all η_p^2 < .04; Figure 7).

Reminder. As in Study 1, during the reminder phase, the main effect of type of CS and all the interactions with type of CS were not significant (all *F*s < 1.14, all *p*s > .33, all η_p^2 < .03).

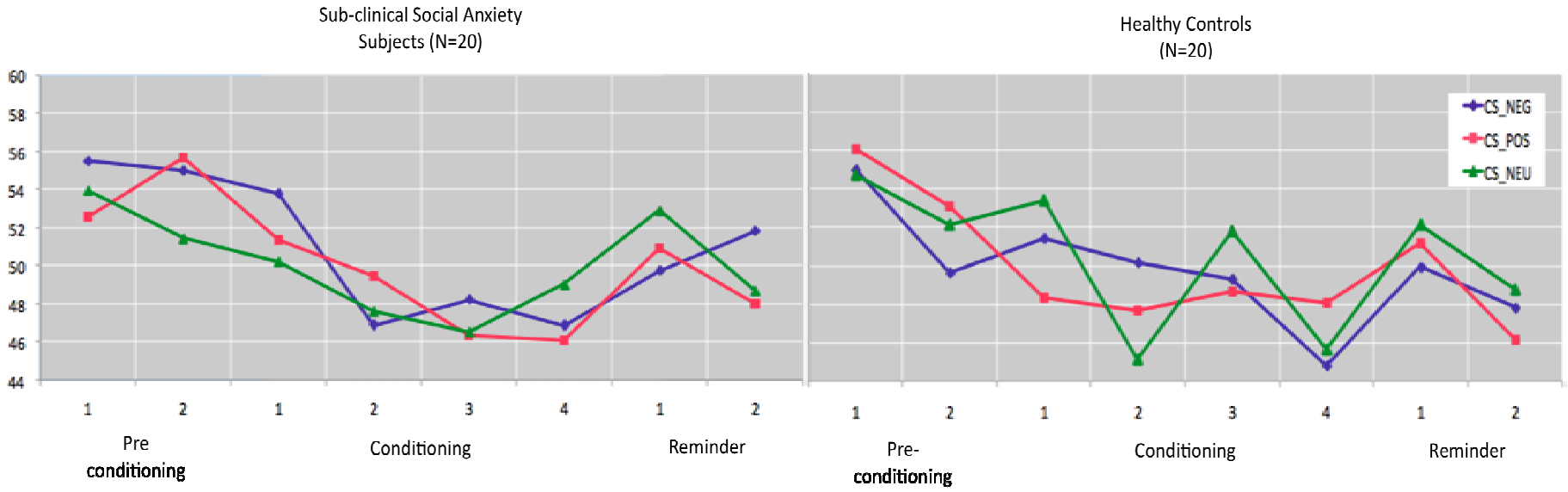

Figure 7. Average, Standardized Startle-Blink EMG Magnitudes During Preconditioning, Conditioning and Reminder phases

Extinction. The main effect of type of CS and all the interactions including type of CS were not significant (all *F*s < 1.44, all *p*s > .21, all η_p^2 < .04).

6.3.2 Self-report reactions to the conditioned stimulus

Preconditioning. As in Study 1, the main effect of type of CS and type of CS by subject group interaction were not significant for all self-report measures (all *F*s < 1.34, all *p*s >.25, all η_p^2 < .04).

Conditioning. As in Study 1, main effects of type of CS were found for the self-report measures of anxiety ($F(2, 72) = 28.27$, $p < .001$, $\eta_p^2 = .44$), unpleasantness ($F(2, 72) = 29.78$, $p < .001$, $\eta_p^2 = .45$) and arousal ($F(2, 58.59) = 23.97$, $p < .001$, $\eta_p^2 = .40$), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for anxiety ($F(1, 36) = 40.16$, $p < .001$, $\eta_p^2 = .53$) and unpleasantness ($F(1, 36) > 49.29$, $p < .001$, $\eta_p^2 > .58$), and a quadratic trend for arousal ($F(1, 36) = 33.40$, $p < .001$, $\eta_p^2 = .48$; see Figure 8). However, as in Study 1, the type of CS by subject group interactions for anxiety, unpleasantness and arousal were not significant (all *F*s < 1.92, all *p*s > .16, all η_p^2 < .05). Again, a posthoc test comparing the CS_{neg} vs. CS_{pos} scores for each subjective variable (anxiety, unpleasantness, and arousal) revealed a significant difference only for the arousal ratings ($F(1, 36) = 5.63$, $p < .02$, $\eta_p^2 = .14$). Pair-wise comparisons indicated higher arousal ratings for the CS_{neg} vs. CS_{pos} in the SSA group ($t(39) = 3.09$, $p = .01$, $d = .49$) but not in the HC group ($t(39) = .28$, $p = 1.00$, $d = .04$).

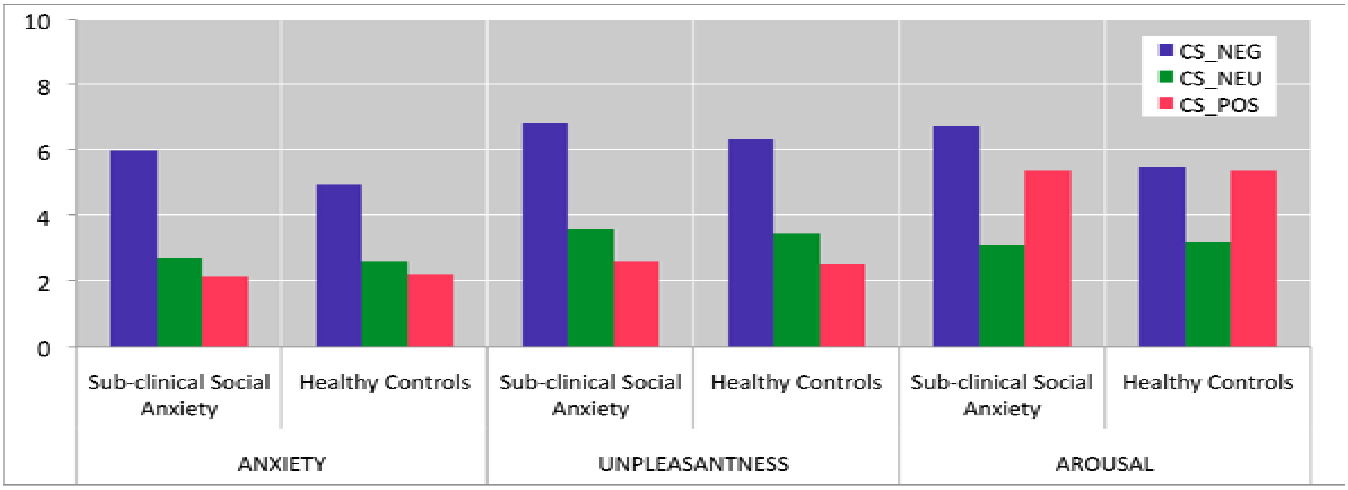

Figure 8. Likert Scale Scores for Self-Report Measures of Anxiety, Unpleasantness, and Arousal During Conditioning

Extinction. Main effects of type of CS were found for the anxiety ($F(2, 72) = 18.44$, $p < .001$, $\eta_p^2 = .34$), unpleasantness ($F(2, 72) = 20.36$, $p < .001$, $\eta_p^2 = .36$), and arousal ratings ($F(2, 72) = 13.43$, $p < .001$, $\eta_p^2 = .27$), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for anxiety ($F(1, 36) = 19.75$, $p < .001$, $\eta_p^2 = .35$) and unpleasantness ($F(1, 36) = 27.10$, $p < .001$, $\eta_p^2 = .43$), and a quadratic trend for arousal ($F(1, 36) = 17.98$, $p < .001$, $\eta_p^2 = .33$; see Figure 9). As in Study 1, the type of CS by subject group interactions for anxiety, unpleasantness and arousal were all not significant (all *F*s < .84, all *p*s > .44, all $\eta_p^2 < .02$).

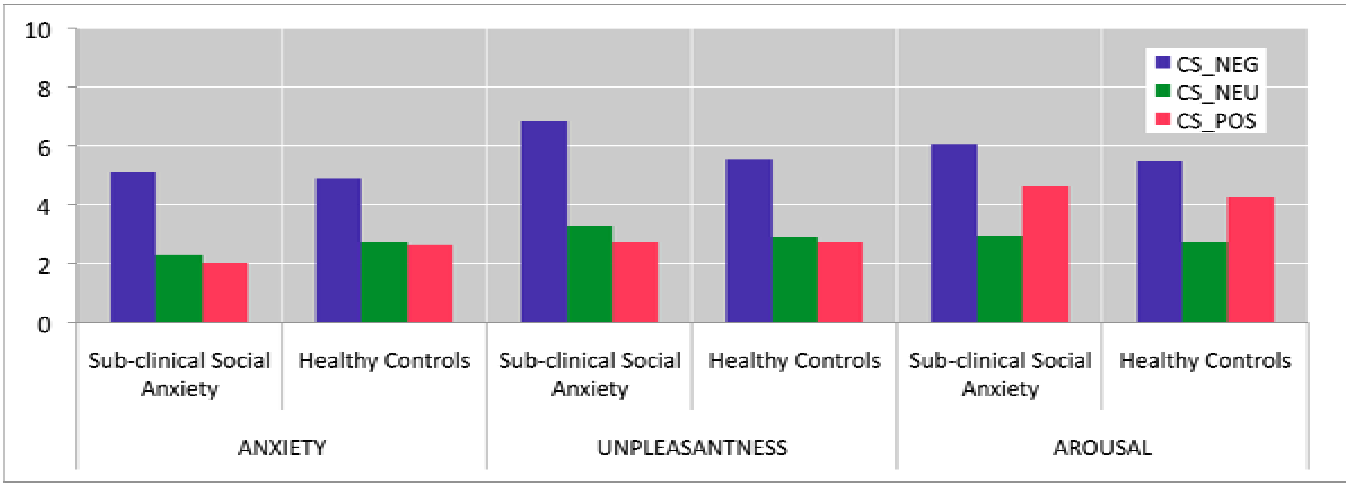

Figure 9. Likert Scale Scores for Self-Report Measures of Anxiety, Unpleasantness, and Arousal During Extinction

6.3.3 Self-report reactions to the unconditioned stimulus

Main effects of type of US were found for the self-report measures of happiness ($F(2, 57.41) = 56.41$, $p < .001$, $\eta_p^2 = .61$) and anxiety ($F(2, 72) = 23.33$, $p < .001$, $\eta_p^2 = .39$), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for the anxiety ratings ($F(1, 36) = 34.02$, $p < .001$, $\eta_p^2 = .49$) and linear decreases from CS_{pos} to CS_{neu} to CS_{neg} for the happiness ratings ($F(1, 36) = 75.12$, $p < .001$, $\eta_p^2 = .68$; see Figure 10). As in Study 1, there were no between groups differences in the happiness or anxiety ratings for the US, as shown by the non-significant type of US by group interactions (both Fs < .1.69, both ps > .19, all $\eta_p^2 < .05$).

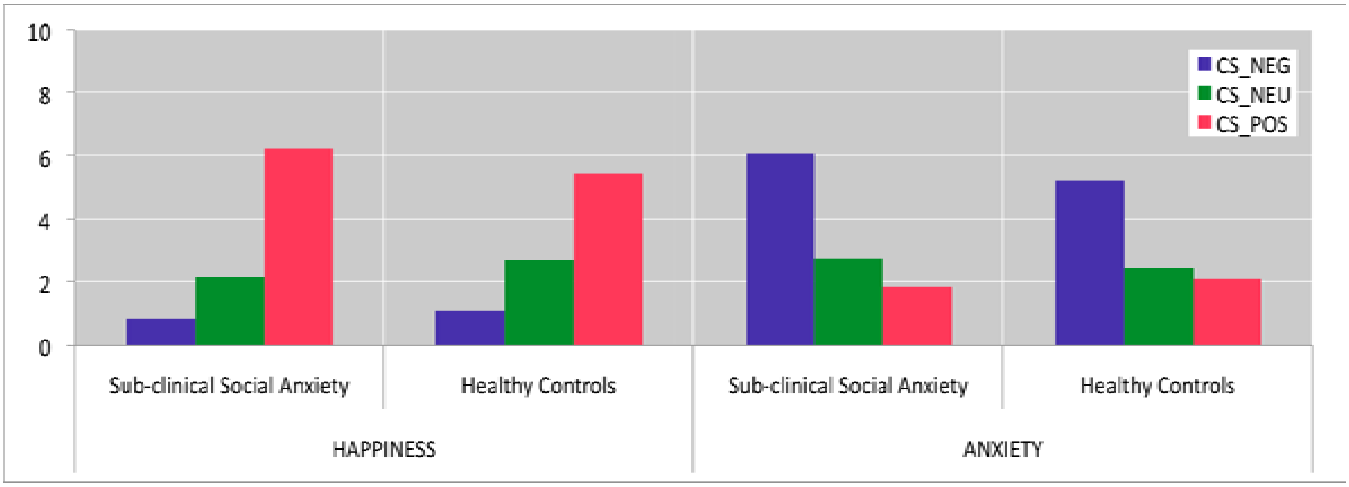

Figure 10. Likert Scale Scores for Self-Report Measures of Happiness and Anxiety to the US

6.4 Correlations between Fear Conditioning and Social Anxiety

None of the Pearson's correlations between the CR (operationalized as CS_{neg}-CS_{pos} during the conditioning phase) and social anxiety questionnaires was significant. For the LSAS, all correlations were not significant in all groups in both studies ($rs < .41$, $ps > .10$). For the FNES, all correlations were not also significant in both studies ($rs < .25$, $ps > .35$).

DISCUSSION AND CONCLUSIONS

7. Discussion

We employed a novel social conditioning paradigm using socially relevant USs to study FC in individuals with social anxiety in two separate studies. In both studies, and for all participant groups, we found evidence of FC for the subjective but not the physiological (i.e., FPS) measures. Contrary to our hypotheses, we found no evidence of enhanced FC (in either subjective or physiological measures) in socially anxious individuals (clinical or sub-clinical) in comparison to healthy controls or to another clinical group (PDA patients). Furthermore, correlations between the CR and scores of social anxiety questionnaires (LSAS and FNES) were all not significant.

7.1. Differences across response systems in Fear Conditioning

In human FC experiments, it is relatively common for conditioning responses to differ across response systems (see Beckers et al., 2013), as shown here. For example, a previous study by Hermann et al. (2002) in SAD patients found evidence of FC for subjective ratings but not for heart rate measures. There are also previous reports of inconsistent results between SCR measurements and subjective ratings (e.g., Tabbert, Stark, Kirsch, & Vaitl, 2006) or neural responses (e.g., Pejic et al., 2011) in non-clinical samples. In the present study, we used FPS as a physiological index of conditioning, and, according to Lipp, Sheridan, & Siddle (1994), the startle reflex can be potentiated as long as the eliciting stimuli is sufficiently aversive. This may suggest that the US employed here were not experienced aversive enough to elicit FPS. However, our anxiety ratings

to the negative US (M=5.13 for Study 1 and M=5.68 for Study 2) were higher than those of Lissek (M=3.97).

7.2 Comparison with previous studies

The fact that in our two studies the SAD (or SSA) individuals did not show enhanced FC compared to healthy controls is at odds with the results from Lissek et al. (2008) and is surprising given that the acquisition phase was identical across our two studies and the study by Lissek and colleagues. Furthermore, our samples of socially anxious participants (both clinical and subclinical) presented similar (or higher) social anxiety severity (SAD: LSAS = 76.56, FNES = 25.31 and SSA: LSAS = 67.80, FNES = 20.25) to those of Lissek (LSAS = 65.11, FNES = 20.16). Additionally, our control individuals also had similar or lower social anxiety severity (LSAS = 17.88, FNES = 8.75 for Study 1 and LSAS = 17.15, FNES = 7.95 for Study 2) as those of Lissek (LSAS = 26.83, FNES = 9.17). Although we included some individuals with non-generalized SAD in our clinical study, excluding these participants did not change the results. In any case, it must be noted that there is little evidence to support the subtyping of SAD (see Stein et al., 2010).

There are, however, some differences between our socially anxious samples and those of Lissek in terms of gender distribution, comorbidity, and ethnicity. As regards gender, the majority (75-80%) of our participants were women, whereas in Lissek's study, the sample was more evenly distributed (55% female). Although, as in Lissek et al. (2008), we did not find a main effect of gender in our analyses, growing evidence

suggests that men and women may diverge in their acquisition of fear (Milad et al., 2006; Inslicht et al., 2012; Lebron-Milad et al., 2012). In fact, a recent study of PTSD patients found greater fear acquisition in women compared to men (see Inslicht et al., 2012). These recent data and the fact that three previous studies on FC and social anxiety with negative results examined only males (Schneider et al., 1999; Hermann et al., 2002; Veit et al., 2002), whereas three studies with positive results included both males and females (Lissek et al., 2008, Davis et al., 2010; Pejic et al., 2011), indicate that gender should be further studied as a possible confounding/explanatory variable in fear acquisition, especially when clinical samples are studied.

With regards to comorbidity, almost 80% of SAD patients in Lissek et al. study (2008) had a current or past comorbid mental disorder, whereas in our study of SAD patients only 19% presented with a comorbid GAD. This raises the possibility that differences between our FPS results and the results of Lissek et al. (2008) are partially explained by the presence of comorbidity. However, the results of a series of recent studies using aversive imagery in ADs (McTeague et al., 2009; McTeague et al., 2010; McTeague, Lang, Laplante, & Bradley, 2011) suggest that comorbidity typically *decreases* FPS and therefore in our "purer" (i.e., with less comorbidity) SAD sample (and also in our SSA sample) the differences in our physiological measures should have been more evident.

In relation to ethnicity, approximately half of Lissek's participants were Caucasian, whereas our entire sample population was Caucasian. Some recent data

suggest that FC may be facilitated and fear extinction may be impaired if CSs of another ethnic group are used (Olsson, Ebert, Banaji, & Phelps, 2005). Furthermore, Fani et al. (2012) have recently stated that faces that represent other ethnic group may act as more arousing and threatening CSs than same-ethnic group CSs and can elicit higher potentiation of the startle reflex. Therefore, one possible explanation for the discrepant findings between our study and Lissek et al. (2008) is that the findings in the latter study, at least in part, reflect a general startle reflex potentiation effect because of the CSs used. This is an important question for future FC studies and further research in this area seems warranted.

It is noteworthy that at least four studies (Schneider et al., 1999; Hermann et al., 2002; Veit et al., 2002), including the present study, have not detected enhanced "conditionability" in SAD individuals in comparison to healthy controls. Although previous research on ADs seems to support the idea that enhanced conditionability is a characteristic of PTSD (e.g., Orr et al., 2000), results regarding other ADs are mixed. For example, at least two previous studies did not find evidence of enhanced conditionability in PD (Del-Ben et al., 2001; Michael et al., 2007) or GAD (Pitman & Orr, 1986), but a recent study using an attitudinal conditioning task found enhanced conditioning in individuals with specific phobias (Vriends, Michael, Schindler, & Margraf, 2011). In some ADs, such as OCD, fear conditioning processes remain largely unexplored. In any case, methodological differences between studies make it difficult to come to conclusions in this area (see Lissek et al., 2005a).

7.3 Other mechanisms involved in SAD

Although, as stated in the introduction, a history of traumatic experiences is common among individuals with SAD, other mechanisms, as opposed to increased fear acquisition, could be "abnormal" in SAD. These include other processes related to fear learning, such as impaired extinction, as well as non-associative processes.

Impaired fear extinction seems to characterize individuals with certain ADs including PTSD (Orr et al., 2000; Peri, et al., 2000; Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007) and PD (Michael et al, 2007). One possibility is that SAD is characterized by a deficit in fear extinction rather than in fear acquisition. To date, only one study has reported that SAD patients show impaired (delayed) extinction (Hermann et al., 2002). In our two studies, we did not find differences in fear extinction between socially anxious individuals (clinical or subclinical) and healthy controls. However, the fact that, as in previous studies (Schneider et al., 1999; Hermann et al., 2002; Veit et al., 2002), we found no evidence of fear acquisition limits the interpretation of our extinction data. In any case, in the only study where SAD individuals showed enhanced fear acquisition (Lissek et al., 2008) there were no between-group differences in extinction. We must also note that our extinction phase was relatively short. Future applications of the paradigm employed here should include a larger extinction phase.

Finally, accounts of SAD that are not based on FC mechanisms exist. An important body of research suggests that cognitive and attentional processes play a major role in the origins or maintenance of pathological social anxiety. For example,

cognitive-behavioral models of SAD suggest that distortions in the processing of social information lead to heightened anxiety in social situations which, in turn, helps to maintain social anxiety (Rapee & Heimberg, 1997; Clark & Wells; 1995). Other theoretical models focus on the attentional bias for threat that seems to characterize SAD patients (Mogg, Phillpot, & Bradley, 2004). The results of the present study suggest that these alternative explanations may have greater weight for understanding social anxiety. However, it must be noted that some recent data from PTSD patients suggest that fear expression may be an effect of *the interaction* between attentional and fear learning processes, rather than an effect of one or the other in isolation (Fani et al., 2012). Future FC studies in social anxiety should therefore also assess attentional processes.

7.4 Limitations

The present study has several limitations. First, our sample sizes were relatively small, albeit similar to previous studies in the field. Second, our participants in the PDA group could be using SSRIs, but previous data suggest that this class of medication does not affect the FPS (Grillon, Chavis, Covington, & Pine, 2009). Finally, as noted above, our paradigm was not specially suited to the study of extinction processes.

8. Conclusions

1. Social anxiety is not related to “abnormal” fear conditioning in individuals with SAD.
2. Social anxiety is not related to “abnormal” fear conditioning in subclinical social anxiety.
3. It is plausible that other associative (e.g. fear extinction) and non-associative processes (e.g cognitive and attentional processes) play a greater role in explaining social anxiety rather than enhanced fear conditioning.

Table 4. Demographic and clinical characteristics of participants in Study 1

	Group[a]								
	Social Anxiety Disorder (*n*=16)		Panic Disorder (*n*=16)		Healthy Controls (*n*=16)				
Variable	Mean	SD	Mean	SD	Mean	SD	F	df	P[d]
Age	25.81	8.50	33.00	8.65	31.06	10.66	2.54	2, 47	ns
LSAS total score (0-144)	76.56_a	22.72	40.13_b	23.29	17.88_c	7.70	37.69	2, 47	<.0001
Anxiety/fear	41.63_a	10.35	20.75_b	11.75	10.50_c	5.41	43.98	2, 47	<.0001
Avoidance	34.94_a	13.16	19.38_b	13.35	7.38_c	3.72	25.11	2, 47	<.0001
FNES (0-30)	25.31_a	4.92	18.31_b	5.64	8.75_c	4.58	43.06	2, 47	<.0001
BDI (0-63)	19.25_a	9.57	16.56_a	9.22	2.01_c	2.51	22.41	2, 47	<.0001
STAI-Trait (0-60)	36.00_a	10.62	29.79_a	10.71	10.81_c	5.94	29.91	2, 43	<.0001
PDSS-SR (0-28)	-	-	12.63	5.89	-	-			
Airpuff rating (0-10)	7.87	4.13	6.25	1.34	6.88	.81	1.65	2, 47	ns
	N	%	N	%	N	%	χ^2	df	
Female gender	13	81.3	11	68.8	13	81.3	0.943	2	ns
Psychiatric comorbidity									
GAD	3	19	0	0	0	0			
Current medication (SSRIs)	0	0	10	62.5	0	0			

LSAS, Liebowitz Social Anxiety Scale; FNES, Fear of Negative Evaluation Scale; BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; PDSS-SR, Panic Disorder Severity Scale self-report; GAD, Generalized Anxiety Disorder; SSRIs, Selective Serotonin Reuptake Inhibitors.

[a]Means with different subscripts differ significantly at p<0.05.

[d]One-way ANOVA (except for gender, which was assessed with a Chi square)

Table 5. Demographic and psychometric characteristics of participants in Study 2

	Sub-clinical Social Anxiety (*n*=20)		Healthy Controls (*n*=20)				
Variable	Mean	SD	Mean	SD	F	df	P[a]
Age	20.15	2.23	21.55	2.60	16.92	1, 39	ns
LSAS total score (0-144)	67.80	13.49	17.15	6.61	227.25	1, 39	<.0001
Anxiety/fear	35.70	7.71	8.85	3.75	196.41	1, 39	<.0001
Avoidance	32.10	7.63	8.30	4.52	144.07	1, 39	<.0001
FNES (0-30)	20.25	6.18	7.95	5.12	46.87	1, 39	<.0001
BDI (0-63)	11.47	9.16	4.50	4.80	9.00	1, 39	p=.005
STAI-Trait (0-60)	23.63	10.28	10.65	4.62	24.60	1, 39	<.0001
Airpuff rating (0-10)	6.45	1.50	4.65	2.56	7.35	1, 39	p=.010
	N	%	N	%	χ^2	df	
Female gender	15	75	15	75	0.00	1	ns

LSAS, Liebowitz Social Anxiety Scale; FNES, Fear of Negative Evaluation Scale; BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; PDSS-SR, Panic Disorder Severity Scale self-report.

[a]Two tailed t-tests (except for gender, which was assessed with a Chi square)

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APPENDICES

LSAS

INSTRUCCIONES

A continuación encontrará unas frases que hacen referencia a distintas situaciones. Por favor, lea las frases e indique con un número de 0 a 3 el grado de miedo o ansiedad que experimenta en cada situación y la frecuencia con que la evita.

Miedo o ansiedad
0. Nada de miedo o ansiedad
1. Un poco de miedo o ansiedad
2. Bastante miedo o ansiedad
3. Mucho miedo o ansiedad

Evitación
0. Nunca lo evito (0%)
1. En ocasiones lo evito (1-33%)
2. Frecuentemente lo evito (33-67%)
3. Habitualmente lo evito (67-100%)

	Miedo / ansiedad	Evitación
1. Llamar por teléfono en presencia de otras personas		
2. Participar en grupos pequeños		
3. Comer en lugares públicos		
4. Beber con otras personas en lugares públicos		
5. Hablar con personas que tienen autoridad		
6. Actuar, hacer una representación o dar una charla ante un público		
7. Ir a una fiesta		
8. Trabajar mientras le están observando		
9. Escribir mientras le están observando		
10. Llamar por teléfono a alguien que usted no conoce demasiado		
11. Hablar con personas que usted no conoce demasiado		
12. Conocer a gente nueva		
13. Orinar en servicios públicos		
14. Entrar en una sala cuando el resto de la gente ya está sentada		
15. Ser el centro de atención		
16. Intervenir en una reunión		
17. Hacer un examen, test o prueba		
18. Expresar desacuerdo o desaprobación a personas que usted no conoce demasiado		
19. Mirar a los ojos a alguien que usted no conoce demasiado		
20. Exponer un informe a un grupo		
21. Intentar «ligarse» a alguien		
22. Devolver una compra a una tienda		
23. Dar una fiesta		
24. Resistir a la presión de un vendedor muy insistente		

STAI-E

A continuación encontrará unas frases que se utilizan corrientemente para describirse uno a sí mismo. Lea cada frase y señale la puntuación de 0 a 3 que indique mejor cómo se **SIENTE Vd. AHORA MISMO, EN ESTE MOMENTO**. No hay respuestas buenas ni malas. No emplee demasiado tiempo en cada frase y conteste señalando la respuesta que mejor describa su situación presente.

	Nada	Algo	Bastante	Mucho
1. Me siento calmado	0	1	2	3
2. Me siento seguro	0	1	2	3
3. Estoy tenso	0	1	2	3
4. Estoy contrariado	0	1	2	3
5. Me siento cómodo (estoy a gusto)	0	1	2	3
6. Me siento alterado	0	1	2	3
7. Estoy preocupado ahora por posibles desgracias futuras	0	1	2	3
8. Me siento descansado	0	1	2	3
9. Me siento angustiado	0	1	2	3
10. Me siento confortable	0	1	2	3
11. Tengo confianza en mí mismo	0	1	2	3
12. Me siento nervioso	0	1	2	3
13. Estoy desasosegado	0	1	2	3
14. Me siento muy "atado" (como oprimido)	0	1	2	3
15. Estoy relajado	0	1	2	3
16. Me siento satisfecho	0	1	2	3
17. Estoy preocupado	0	1	2	3
18. Me siento aturdido y sobreexcitado	0	1	2	3
19. Me siento alegre	0	1	2	3
20. En este momento me siento bien	0	1	2	3

COMPRUEBE SI HA CONTESTADO A TODAS LAS FRASES CON UNA SOLA RESPUESTA

STAI-R

A continuación encontrará unas frases que se utilizan corrientemente para describirse uno a sí mismo. Lea cada frase y señale la puntuación 0 a 3 que indique mejor cómo se **SIENTE Vd. EN GENERAL**, en la mayoría de las ocasiones. No hay respuestas buenas ni malas. No emplee demasiado tiempo en cada frase y conteste señalando lo que mejor describa cómo se siente Vd. generalmente.

	Casi nunca	A veces	A menudo	Casi siempre
21. Me siento bien	0	1	2	3
22. Me canso rápidamente	0	1	2	3
23. Siento ganas de llorar	0	1	2	3
24. Me gustaría ser tan feliz como otros	0	1	2	3
25. Pierdo oportunidades por no decidirme pronto	0	1	2	3
26. Me siento descansado/a	0	1	2	3
27. Soy una persona tranquila, serena y sosegada	0	1	2	3
28. Veo que las dificultades se amontonan y no puedo con ellas	0	1	2	3
29. Me preocupo demasiado por cosas sin importancia	0	1	2	3
30. Soy feliz	0	1	2	3
31. Suelo tomar las cosas demasiado seriamente	0	1	2	3
32. Me falta confianza en mí mismo/a	0	1	2	3
33. Me siento seguro/a	0	1	2	3
34. No suelo afrontar las crisis o dificultades	0	1	2	3
35. Me siento triste (melancólico/a)	0	1	2	3
36. Estoy satisfecho/a	0	1	2	3
37. Me rondan y molestan pensamientos sin importancia	0	1	2	3
38. Me afectan tanto los desengaños, que no puedo olvidarlos	0	1	2	3
39. Soy una persona estable	0	1	2	3
40. Cuando pienso sobre asuntos y preocupaciones actuales, me pongo tenso/a y agitado/a	0	1	2	3

COMPRUEBE SI HA CONTESTADO A TODAS LAS FRASES CON UNA SOLA RESPUESTA

FNES

INSTRUCCIONES

Por favor conteste si está de acuerdo o no con las siguientes afirmaciones. Verdadero corresponderá a que sí está de acuerdo y Falso corresponderá a que no está de acuerdo. V = verdadero F = falso

	V	F
1. Casi nunca me preocupa parecer tonto ante los demás.		
2. Me preocupa lo que la gente pensará de mí, incluso cuando sé que no me creará ningún problema.		
3. Me pongo tenso y nervioso si sé que alguien me está analizando/evaluando.		
4. No me preocupa saber si la gente está formándose una impresión desfavorable de mí.		
5. Me siento muy afectado cuando cometo algún error en una situación social.		
6. Las opiniones que la gente que considero importante tiene de mí me causan poca ansiedad.		
7. Temo a menudo que pueda parecer ridículo o hacer alguna tontería.		
8. Apenas sé como reaccionar cuando otras personas me censuran.		
9. Temo a menudo que la gente se dé cuenta de mis defectos.		
10. La desaprobación de los demás podría tener poco efecto sobre mí.		
11. Si alguien me está evaluando, tiendo a esperar lo peor.		
12. Raramente me preocupo de la impresión que estoy causando en alguna persona.		
13. Tengo miedo de que otros no aprueben mi conducta.		
14. Me da miedo que la gente me critique.		
15. Las opiniones de los demás sobre mí no me preocupan.		
16. No me siento necesariamente afectado si no le caigo bien a alguien.		
17. Cuando estoy hablando con alguien, me preocupa lo que pueda estar pensando acerca de mí.		
18. Creo que se pueden cometer errores sociales algunas veces. Entonces ¿por qué preocuparme?		
19. Generalmente me preocupa la impresión que pueda causar.		
20. Me preocupa bastante lo que mis jefes piensen de mí.		
21. Si sé que alguien me está juzgando, esto tiene poco efecto sobre mí.		
22. Me preocupa que los demás piensen que no valgo la pena.		
23. Me preocupa poco lo que los demás puedan pensar de mí.		
24. A veces pienso que estoy demasiado preocupado por lo que otras personas piensan de mí.		
25. A menudo me preocupa que pueda decir o cometer equivocaciones.		
26. A menudo soy indiferente acerca de las opiniones que los demás tienen de mí.		
27. Generalmente confío en que los demás tendrán una impresión favorable de mí.		
28. A menudo me preocupa que la gente que me es importante no piense muy favorablemente de mí.		
29. Me obsesiono por las opiniones que mis amigos tienen de mí.		
30. Me pongo tenso y nervioso si sé que estoy siendo juzgado por mis jefes.		

BDI-II FECHA:

Nombre:.....Edad:.....Sexo:.....
Estado Civil:.....Profesión:.....Estudios:.....

INSTRUCCIONES: Este cuestionario consiste en 21 grupos de afirmaciones. Por favor, lea con atención cada uno de ellos y, a continuación, señale cuál de las afirmaciones de cada grupo describe mejor el modo en el que se ha sentido **DURANTE LAS DOS ÚLTIMAS SEMANAS**, **INCLUYENDO EL DÍA DE HOY**. Rodee con un círculo el número que se encuentre escrito a la izquierda de la afirmación que haya elegido. Si dentro del mismo grupo, hay más de una afirmación que considere igualmente aplicable a su caso, señálela también. **Asegúrese de leer todas las afirmaciones dentro de cada grupo antes de efectuar la elección.**

1. Tristeza

0 No me siento triste habitualmente.
1 Me siento triste gran parte del tiempo.
2 Me siento triste continuamente.
3 Me siento tan triste o tan desgraciado que no puedo soportarlo.

2. Pesimismo

0 No estoy desanimado sobre mi futuro.
1 Me siento más desanimado sobre mi futuro que antes.
2 No espero que las cosas mejoren.
3 Siento que mi futuro es desesperanzador y que las cosas sólo empeorarán.

3. Sentimientos de Fracaso

0 No me siento fracasado.
1 He fracasado más de lo que debería.
2 Cuando miro atrás, veo fracaso tras fracaso.
3 Me siento una persona totalmente fracasada.

4. Pérdida de Placer

0 Disfruto de las cosas que me gustan tanto como antes.
1 No disfruto de las cosas tanto como antes.
2 Obtengo muy poco placer de las cosas con las que antes disfrutaba.
3 No obtengo ningún placer de las cosas con las que antes disfrutaba.

5. Sentimientos de Culpa

0 No me siento especialmente culpable.
1 Me siento culpable de muchas cosas que he hecho o debería haber hecho.
2 Me siento bastante culpable la mayor parte del tiempo.
3 Me siento culpable constantemente.

6. Sentimientos de castigo

0 No siento que esté siendo castigado.
1 Siento que puedo ser castigado.
2 Espero ser castigado.
3 Siento que estoy siendo castigado

7. Insatisfacción con uno mismo.

0 Siento lo mismo que antes sobre mí mismo.
1 He perdido confianza en mí mismo.
2 Estoy decepcionado conmigo mismo.
3 No me gusto.

8. Auto-Críticas

0 No me critico o me culpo más que antes.
1 Soy más crítico conmigo mismo de lo que solía ser.
2 Critico todos mis defectos.
3 Me culpo por todo lo malo que sucede.

9. Pensamientos o Deseos de Suicidio

0 No tengo ningún pensamiento de suicidio.
1 Tengo pensamientos de suicidio, pero no los llevaría a cabo.
2 Me gustaría suicidarme.
3 Me suicidaría si tuviese la oportunidad.

10. Llanto

0 No lloro más de lo que solía hacerlo.
1 Lloro más de lo que solía hacerlo.
2 Lloro por cualquier cosa.
3 Tengo ganas de llorar continuamente, pero no puedo.

_____ Puntuación Página 1

CONTINÚA EN LA PÁGINA SIGUIENTE⇒

11. Agitación

0 No estoy más inquieto o agitado que de costumbre.
1 Me siento más inquieto o agitado que de costumbre.
2 Estoy tan inquieto o agitado que me cuesta estarme quieto.
3 Estoy tan inquieto o agitado que tengo que estar continuamente moviéndome o haciendo algo.

12. Pérdida de Interés

0 No he perdido el interés por otras personas o actividades.
1 Estoy menos interesado que antes por otras personas o actividades.
2 He perdido la mayor parte de mi interés por los demás o por las cosas.
3 Me resulta difícil interesarme en algo.

13. Indecisión

0 Tomo decisiones más o menos como siempre.
1 Tomar decisiones me resulta más difícil que de costumbre.
2 Tengo mucha más dificultad en tomar decisiones que de costumbre.
3 Tengo problemas para tomar cualquier decisión.

14. Inutilidad

0 No me siento inútil.
1 No me considero tan valioso y útil como solía ser.
2 Me siento inútil en comparación con otras personas.
3 Me siento completamente inútil.

15. Pérdida de Energía

0 Tengo tanta energía como siempre.
1 Tengo menos energía de la que solía tener.
2 No tengo suficiente energía para hacer muchas cosas.
3 No tengo suficiente energía para hacer nada.

16. Cambios en el Patrón de Sueño.

0 No he experimentado ningún cambio en mi patrón de sueño.

1a Duermo algo más de lo habitual.
1b Duermo algo menos de lo habitual.

2a Duermo mucho más de lo habitual.
2b Duermo mucho menos de lo habitual.

3a Duermo la mayor parte del día.
3b Me despierto 1 o 2 horas más temprano y no puedo volver a dormirme.

17. Irritabilidad

0 No estoy más irritable de lo habitual.
1 Estoy más irritable de lo habitual.
2 Estoy mucho más irritable de lo habitual.
3 Estoy irritable continuamente.

18. Cambios en el Apetito

0 No he experimentado ningún cambio en mi apetito.

1a Mi apetito es algo menor de lo habitual.
1b Mi apetito es algo mayor de lo habitual.

2a Mi apetito es mucho menor que antes.
2b Mi apetito es mucho mayor de lo habitual.

3a He perdido completamente el apetito.
3b Tengo ganas de comer continuamente.

19. Dificultad de Concentración

0 Puedo concentrarme tan bien como siempre.
1 No puedo concentrarme tan bien como habitualmente.
2 Me cuesta mantenerme concentrado en algo durante mucho tiempo.
3 No puedo concentrarme en nada.

20. Cansancio o Fatiga

0 No estoy más cansado o fatigado que de costumbre.
1 Me canso o fatigo más fácilmente que de costumbre.
2 Estoy demasiado cansado o fatigado para hacer muchas cosas que antes solía hacer.
3 Estoy demasiado cansado o fatigado para hacer la mayoría de las cosas que antes solía hacer.

21. Pérdida de Interés en el Sexo

0 No he notado ningún cambio reciente en mi interés por el sexo.
1 Estoy menos interesado por el sexo de lo que solía estar.
2 Estoy mucho menos interesado por el sexo ahora.
3 He perdido completamente el interés por el sexo.

_____ Puntuación Página 1

_____ Puntuación Página 2

_____ PUNTUACIÓN TOTAL (Págs. 1+2)

ESCALA DE GRAVEDAD DEL TRASTORNO DE PÁNICO AUTOAPLICADA (PDSS-SR)

Desarrollada y calibrada por M. Katherine Shear M.D. y David A. Spiegel M.D.
Versión española desarrollada por Dr. A. Bulbena, adaptada por R. Delgado

Nombre …… Fecha …… /……/……

Algunas de las preguntas siguientes se refieren a ataques de pánico o bien a ataques con síntomas limitados. A efectos de este cuestionario definimos un *ataque de pánico* como una **aparición brusca** de malestar o miedo acompañado de **por lo menos 4 de los síntomas que aparecen en la lista a continuación**. Para que se considere como ataque, los síntomas deben **llegar al máximo en el plazo de unos 10 minutos**. Los ataques semejantes a los ataques de pánico pero con menos de cuatro de los síntomas de la lista se denominan *ataques con síntomas limitados* (ASL).
A continuación tiene los síntomas:

- · Frecuencia cardíaca aumentada o sacudidas del corazón
- · Sudoración
- · Temblores o sacudidas
- · Sensación de ahogo o falta de
- · Sensación de atragantarse
- · Opresión o malestar en el pecho
- · Náusea
- · Mareo o inestabilidad
- · Sensación de irrealidad
- · Entumecimiento u hormigueo
- · Escalofríos o sofocaciones
- · Miedo de perder el control volverse loco
- · Miedo a morir

En cada una de las siguientes preguntas, marque por favor la respuesta que mejor describa lo que ha experimentado en el **último mes**.

1. ¿Cuántos ataques de pánico o ataques con síntomas limitados ha tenido en el último mes?

   0. Ningún ataque de pánico ni ataque con síntomas limitados
   1. Ligero: Ningún ataque de pánico completo y no más de 1 ataque con síntomas limitados/día
   2. Moderado: 1 o 2 ataques de pánico completos y/o múltiples ataques con síntomas limitados/día
   3. Grave: Más de 2 ataques de pánico completos, pero no más de 1/día de promedio
   4. Extremo: Más de un ataque de pánico completo al día, la mayoría de días

2. Si usted ha padecido algún ataque de pánico durante el último mes, ¿qué grado de malestar (incomodidad, miedo, etc) sintió **durante los ataques**? (Si sintió más de uno, ponga el promedio). Si no ha tenido ningún ataque de pánico pero sí ataques con síntomas limitados, conteste la pregunta en relación a éstos.

   0. Ningún malestar, o ningún ataque de pánico ni ataques con síntomas limitados en el último mes

1. Malestar ligero (no muy intenso)
2. Malestar moderado (intenso, pero todavía soportable)
3. Malestar grave (muy intenso)
4. Malestar extremadamente grave (malestar extremo durante todos los ataques)

3. Durante el último mes, ¿hasta qué punto se ha sentido usted preocupado/a o ansioso/a, **temiendo un nuevo ataque o por miedos relacionados con los ataques** (por ejemplo, por si tener ataques significara que tiene problemas físicos o mentales o por si tener ataques hiciera que los demás lo/a valoraran negativamente)?

0. Nada preocupado/a
1. Ocasionalmente o de manera ligera
2. Frecuentemente o de forma moderada
3. Muy a menudo o en un grado muy molesto
4. Casi constantemente o en un grado incapacitante

4. Durante el último mes, ¿ha habido **lugares o situaciones** (ej. transporte público, cines, teatros, aglomeraciones, puentes, túneles, grandes almacenes, quedarse solo/a) en los que usted haya sentido miedo o estado ansioso/a (con ganas de marcharse o salir) o que usted haya evitado **por miedo a tener un ataque de pánico**? ¿Existen otras situaciones en que habría sentido miedo o que habría evitado por este motivo si se hubieran dado durante el último mes? Si su respuesta es afirmativa, puntúe el grado de miedo y evitación durante el último mes.

0. Ningún miedo ni evitación
1. Ligero: Miedo ocasional y/o evitación, pero habitualmente he podido afrontar o aguantar la situación. No ha habido o apenas ha habido cambios en mi día a día
2. Moderado: Miedo y/o evitación marcados, pero soportables. Evité situaciones temidas pero pude afrontarlas acompañado/a. Ha habido algunos cambios en mi día a día, pero mi funcionamiento general no se ha visto afectado
3. Grave: Evitación amplia. He tenido que cambiar mucho mi día a día para adaptarme a la evitación, lo que me ha dificultado hacer mis actividades habituales
4. Extremo: Miedo y/o evitación generalizados e incapacitantes. Ha sido necesario cambiar mucho mi día a día hasta el punto que he dejado de hacer cosas importantes

5. Durante el último mes, ¿ha habido **actividades** que ha evitado o en las que ha sentido miedo (malestar), **porque le producían sensaciones parecidas a las de los ataques de pánico o porque pudieran desencadenar un ataque**? (ej. tomar café, hacer ejercicio físico, tener relaciones sexuales, tomar una ducha con agua caliente, ver una película muy emocionante o de terror, etc). ¿Existen otras actividades en que habría sentido miedo o que habría evitado por este motivo si se hubieran dado durante el último mes? Si su respuesta es afirmativa, puntúe el grado de temor y evitación de estas actividades durante el último mes.

0. Ningún miedo ni evitación de situaciones o actividades debido a las sensaciones físicas desagradables
1. Ligero: Miedo ocasional y/o evitación, pero habitualmente he podido tolerar con poco malestar situaciones que provocaran sensaciones físicas. Apenas ha habido cambios en mi día a día
2. Moderado: Evitación marcada pero soportable. Ha habido claros, pero limitados, cambios en mi día a día de forma que mi funcionamiento general no se ha visto afectado
3. Grave: Evitación amplia. Mi día a día ha cambiado sustancialmente o la evitación ha interferido en mi funcionamiento
4. Extremo: Evitación acentuada e incapacitante. Ha habido muchos cambios en mi día a día hasta el punto que he dejado de hacer cosas importantes

6. Durante el último mes, ¿hasta qué punto todos estos síntomas (ataques de pánico o ataques con síntomas limitados, preocupación por los ataques y miedo a situaciones o actividades debido a los ataques) han interferido en su **trabajo o sus responsabilidades domésticas**? (si su trabajo o sus responsabilidades domésticas fueron menores de lo habitual durante el último mes, conteste cómo hubieran interferido en una época de actividad habitual).

0. Ninguna interferencia en el trabajo o responsabilidades domésticas
1. Ligera interferencia en el trabajo o responsabilidades domésticas, pero he podido hacer casi todo lo mismo que si no hubiera tenido estos problemas
2. Interferencia significativa en el trabajo o responsabilidades domésticas, pero he podido hacer las cosas que tenía que hacer
3. Deterioro sustancial en mi rendimiento laboral o doméstico, hasta el punto que ha habido muchas cosas importantes que no he podido hacer debido a estos problemas
4. Deterioro extremo, incapacitante, hasta el punto que he sido incapaz de trabajar o hacer las cosas de casa

7. Durante el último mes, ¿hasta qué punto todos estos síntomas (ataques de pánico o ataques con síntomas limitados, preocupación acerca de ello y evitación de situaciones o actividades) han interferido en su **actividad social**? (si no ha tenido oportunidad de estar con gente el último mes, conteste cómo hubiera interferido si la hubiera tenido).

0. Ninguna interferencia
1. Ligera interferencia en las actividades sociales, pero he podido hacer casi todo lo mismo que si no hubiera tenido estos problemas
2. Interferencia significativa en las actividades sociales, pero he podido hacer la mayoría de cosas esforzándome
3. Deterioro sustancial de mis actividades sociales, hasta el punto que ha habido muchas actividades sociales que no he podido hacer debido a estos problemas
4. Deterioro extremo, incapacitante hasta el punto que me ha resultado casi imposible tener actividad social

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Fear conditioning to socially relevant stimuli is not enhanced in social anxiety disorder or sub-clinical social anxiety

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ABSTRACT

Background and Objectives. Although enhanced fear conditioning has been implicated in the origins of social anxiety disorder (SAD), laboratory evidence in support of this association is limited. Using a recently developed paradigm employing socially relevant unconditioned stimuli, we conducted two separate studies to assess fear conditioning in individuals with SAD and in non-clinical individuals with high social anxiety (SSA). *Methods*. Individuals with SAD and SSA were compared to age and gender-matched individuals with another anxiety disorder (panic disorder with agoraphobia, PDA) and to healthy controls (Study 1) and with healthy controls (Study 2) *Results*. Contrary to our expectations, in both studies the physiological (fear-potentiated startle) and self-report measures of fear conditioning failed to discriminate between SAD, SSA and the other participant groups. *Limitations*. Patients with PDA could be taking selective serotonin reuptake inhibitors; our paradigm was not specially suited to study fear extinction. *Conclusions*. Enhanced fear conditioning does not seem to play a major role in pathological social anxiety.

Keywords: Fear Conditioning; Social Anxiety Disorder; Panic Disorder; Fear-Potentiated Startle; Anxiety Disorders.

1. Introduction

Fear conditioning is a form of associative learning in which an aversive unconditioned stimulus (US) is repeatedly paired with a neutral conditioned stimulus (CS), resulting in a conditioned fear response. Fear conditioning has long been considered a central pathogenic pathway in anxiety disorders (Lissek et al., 2005; Mineka & Zinbarg, 2006).

Social anxiety disorder (SAD) is a common anxiety disorder characterized by a persistent irrational fear of social/performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others (American Psychiatric Association, 2000). Fear conditioning may play a significant role in SAD. For example, a highly humiliating public speaking experience (US) could be associated with certain people or places (CS) and contribute to the persistence of social anxiety beyond the presence of the US in SAD individuals. Although some data suggest that abnormal (i.e., increased) fear conditioning may be involved in SAD, most of the evidence regarding the role of fear conditioning in SAD is based on subjective and retrospective data (see Lissek et al., 2008). In fact, at least three laboratory studies have failed to show enhanced conditionability in SAD patients compared to healthy controls. These studies used different psychophysiological indexes of fear conditioning, including skin conductance, fear-potentiated startle (FPS) or heart rate, and general aversive US, such as unpleasant odors or painful pressure (Schneider et al., 1999; Hermann, Ziegler, Birbaumer & Flor, 2002; Veit et al., 2002). This lack of evidence poses a major problem to the fear conditioning model of SAD.

However, a recently developed, ecologically enhanced social conditioning

paradigm with socially relevant stimuli as the US showed enhanced fear conditioning in SAD patients. In this study, Lissek et al. (2008) compared individuals with SAD and healthy controls (HC) in a differential fear conditioning paradigm using negative insults with critical faces as the US and neutral faces as the CS. Only individuals with SAD displayed evidence of fear conditioning to the CS. Two recent neuroimaging studies in healthy subjects using socially relevant US also found that social anxiety was associated with enhanced fear conditioning (Davis et al., 2010; Pejic, Hermann, Vaitl & Stark, 2011).

The study by Lissek et al. (2008) also highlighted the importance of using disorder-specific US in the study of fear conditioning in anxiety disorders. It is relatively well established that the qualitative nature of the conditioned response (CR) depends on the qualitative nature of the CS and, therefore, that the closer and more similar the CS is to the US, the stronger the CR will be (see Bouton, Mineka & Barlow, 2001). This "specificity hypothesis" implies that using socially relevant stimuli as the US should elicit "higher conditioning" in SAD compared to other anxiety patients (in the same manner, interoceptive cues should elicit "higher conditioning" in panic disorder versus other anxiety disorders; see De Cort, Griez, Büchler & Schruers, 2012). However, determining whether this enhanced conditionability to socially relevant US is specific to SAD in comparison to other anxiety disorders remains to be tested.

A limitation in the use of clinical populations for studying fear conditioning in patients with anxiety disorders is frequent comorbidity among the different anxiety disorders and between the anxiety disorders and other mental disorders (Merikangas & Swanson, 2010). For example, almost 40% of the SAD individuals in Lissek et al. (2008)

had a current comorbid anxiety disorder. One possibility for circumventing this limitation is the use of sub-clinical samples ("at-risk" individuals with significant social anxiety symptoms but who do not fulfill the criteria for SAD). This seems justified given epidemiological data showing that SAD exists along a continuum (Bögels et al., 2010; Stein, Torgrud & Walker, 2000).

To address these questions, we conducted two separate studies using the new paradigm developed by Lissek et al. (2008). In Study 1, we compared fear conditioning across two clinical groups (SAD and panic disorder with agoraphobia, PDA) and HC. We expected SAD individuals to show greater fear conditioning than individuals with PDA and HC. To our knowledge, this is the first attempt to replicate the Lissek et al. study (differences between SAD patients and HC) and the first test of the "specificity hypothesis" (differences between SAD and PDA patients). In Study 2, we tested whether enhanced fear conditioning was also evident in individuals "at-risk" for SAD. To this end, we compared social conditioning across individuals with sub-clinical social anxiety (SSA) and HC. We expected individuals with SSA to show greater levels of fear conditioning than the HC.

2. Method

2.1 Participants

Approximately 700 university students from two universities from Barcelona (Spain) were screened using the self-report version of the Liebowitz Social Anxiety Scale (LSAS-SR, Bobes, 1999) via a web system. On the basis of their LSAS-SR scores, 120 of these individuals were interviewed by a licensed clinical psychologist not involved in the

experimental phase using the Spanish version of the Mini International Neuropsychiatric Interview (MINI, Sheehan et al., 1998). The MINI has shown to have have good concordance with other diagnostic measures, and good interrater and test-retest reliability (Sheehan et al., 1998; Lecubrier et al., 1997). In a previous study in a Spanish sample, the sensitivity of SAD diagnosis was 100 and its specificity was 84,2 (Bobes, 1998).

Participants who fulfilled the inclusion/exclusion criteria and agreed to participate in the experimental phase (n=68) were allocated to one of the following groups: SAD (n=12, LSAS>50 and current SAD diagnosis) or HC (n=16, LSAS<20, and no current mental disorder) (Study 1) and SSA (n=20, LSAS>50 and no current mental disorder) or HC (n=20, LSAS<20, and no current mental disorder) (Study 2). The HC for each study were selected from the initial screening sample based on their LSAS score and optimal matching to the SAD/PDA groups or to the SSA group with regards to age and gender. Four additional SAD participants and all the participants in the PDA group (n=16, current PDA diagnosis) in Study 1 were recruited from the Anxiety Unit of the Hospital del Mar (Barcelona, Spain). The same clinical psychologist using the MINI also assessed these participants.

Of the 16 participants with SAD, 12 met the criteria for generalized SAD and 4 met the criteria for non-generalized SAD[1]. The exclusion criteria for all participants included: 1) use of pharmacological medication (except for the PDA group, see below) or presence of medical pathology capable of interfering with the study objectives, 2) use of illicit drugs, 3) pregnancy and 4) not speaking Spanish. Additional exclusion criteria for

[1] We operationalized "generalized SAD" as having clinically significant anxiety (fear or avoidance score=3) in at least four different social situations, as assessed by the LSAS. The other participants were classified in the "nongeneralized SAD" group.

the SAD and PDA subjects included: 1) current or past history of bipolar disorder, psychosis or delusional disorder, 2) current major depressive disorder, post-traumatic stress disorder or suicidal ideation, 3) alcohol or substance abuse or dependence (other than nicotine) during the last 3 months and 4) psychomotor delay. Participants in the PDA group could be taking antidepressants (selective serotonin reuptake inhibitors) provided that they had been on a stable dose for at least 3 months prior to the study. All participants were Caucasian.

2. 2 Procedure

We used the paradigm developed by Lissek et al. (2008), with slight modification (adding a reminder phase and using a shorter extinction phase). The subjects participated in a differential fear conditioning procedure in which 8 s neutral facial expressions from three female actors (blonde, brunette, redhead) served as the CS and were paired with one of three types of a 3 s, 85 dB audiovisual US: 1) insults and critical facial expressions (US_{neg}); 2) comments and neutral facial expressions (US_{neu}); and 3) compliments and positive facial expressions (US_{pos}). The conditioned stimuli paired with the negative, neutral, and positive USs will be referred to as $CS_{neg,}$ $CS_{neu,}$ and $CS_{pos,}$ respectively.

The experiment consisted of four phases: *preconditioning* - 4 presentations of each type of CS in absence of the US; *conditioning* - 8 presentations of each type of CS immediately paired with its respective US; *reminder* - 4 presentations of each type of CS immediately paired with its respective US and *extinction* - 4 presentations of each type of CS in absence of the US. The preconditioning and conditioning phases were identical to those of Lissek et al. (2008). The reminder phase followed a 5 min break during which

participants filled out the questionnaires. The reminder phase was added to ensure intact conditioning performance at the start of the extinction phase. The stimuli were presented in a quasi-random order, with the constraint of a maximum of two equal consecutive presentations. The assignment of faces to a type of CS was counterbalanced across subjects.

During the experimental session all the participants completed the Spanish versions of the State Trait Anxiety Inventory (STAI; Spielberger, Gorsuch & Lushene, 1982), the LSAS-SR[2], the Fear of Negative Evaluation Scale (FNES; Villa, 1999) and the Beck Depression Inventory-II (BDI-II; Sanz et al., 2005). In addition, the PDA patients completed the Spanish version of the self-report version of the Panic Disorder Severity Scale (PDSS-SR; Houck, Spiegel, Shear & Rucci., 2002).

All the participants were paid 15€ and, after the experimental procedure was described in detail, were asked to sign an informed consent form, which was previously approved by the corresponding institution's Ethical Research Committee.

2.3 Physiological recordings and subjective ratings

A BIOPAC Mod. MP150WSW psychophysiological recording system was used for stimulation and recording. The startle probe consisted of a 40 ms, 3.5 psi airpuff delivered through a polyethylene tube affixed 1 cm from the participants' foreheads. The startle blink reflex was recorded electromyographically (EMG) using two 5 mm silver surface electrodes placed under the left eye. Impedance levels were maintained below 5 KΩ. The EMG signals were amplified using a 10-500 Hz bandpass analog filter and were digitalized at a rate of 2000 Hz.

The experimental phase began with 9 startle probes. During all four phases, the startle probes were delivered 4 or 5 s after CS onset. An 18-25 s interprobe interval (ITI) was maintained throughout the whole experiment. Ratings of anxiety, unpleasantness and arousal for the CS were obtained from participants on a 10-point Likert scale following conditioning and extinction. Additionally, between the conditioning and reminder phases, the participants rated each type of US by reflecting on the level of anxiety and happiness elicited using a 10-point Likert scale.

2. 4 Data analysis

The startle EMG data were rectified and smoothed within a 20 ms moving window. The blink onset latency window was 20-100 ms and the peak magnitude was determined within 150 ms following response onset. The average EMG baseline levels for the 50 ms preceding the startle probe were subtracted from the peak magnitude. The EMG magnitudes were standardized using within subject T-score conversions to normalize the data. The conditioning phase was divided into 4 blocks, and the preconditioning, reminder and extinction phases were divided in 2 blocks each, one for each type of conditioned stimuli. For FPS measures, fear conditioning was operationalized as the difference between the CS_{neg} and the CS_{pos} during conditioning and fear extinction, and as the difference between the CS_{neg} and the CS_{pos} in early (first block) versus late (last block) extinction.

In both studies, the EMG data were analyzed using analyses of variance (GLM procedure). In Study 1, we used a 3x2x3x4 design for the conditioning phases and a 3x2x3x2 design for the preconditioning, reminder and extinction phases, where subject

[2] Data presented in Tables 1 and 2 correspond to the second administration of the LSAS-SR

group (SAD, PDA, HC) and gender were the between subject factors, and type of CS (negative, neutral, positive) and time (block 1, 2, 3 and 4 for conditioning; block 1 and 2 for preconditioning, reminder and extinction) were the within-subject factors. In Study 2, we used a 2x2x3x4 design for the conditioning phase and a 2x2x3x2 design for the preconditioning, reminder and extinction phases. Subject group (SSA, HC) and gender were the between-subject factors, and type of CS (negative, neutral, positive) and time (block 1, 2, 3 and 4 for conditioning; block 1 and 2 for preconditioning, reminder and extinction) were the within-subject factors. In both studies, simple contrasts were calculated to specify the main or interaction effects.

Self-report reactions to the conditioned stimuli (anxiety, unpleasantness and arousal) were also analyzed using a GLM procedure with a 3x2x3 (Study 1) or 2x2x3 (Study 2) design. Subject group and gender were the between-subject factors, and type of CS was the within-subject factor.

Eta squared values or Cohen's *d* were calculated as measures of effect size. Gender results are not presented given that the main and interaction effects of gender were not significant for either EMG or the self-report data in both experiments. Since the BDI, STAIT-T and airpuff ratings were not found to interact with any of the dependent measures (data not shown), these effects are not reported below.

3. Results

3.1 Study 1

The demographic and clinical characteristics of the participants in Study 1 are reported in Table 1.

3.1.1 Startle EMG

Preconditioning. The main effects of type of CS, type of CS by subject group and type of CS by time interaction were not significant (all *F*s < 1.75, all *p*s > .30, all η_p^2 < .08).

Conditioning. Although a type of CS by time interaction emerged for the whole sample ($F(6, 234) = 2.28$, $p = .04$, $\eta_p^2 = .06$), follow-up analyses showed that the responses to the CS_{neg} and CS_{pos} were not different in any block during conditioning (all *p*s > .54). Furthermore, the main effect of type of CS and the interactions type of CS by subject group and type of CS by time by subject group were not significant (all *F*s < 1.59, all *p*s > .19, all η_p^2 < .08; Figure 1).

Reminder. During the reminder phase, the main effect of type of CS and all the interactions with type of CS were not significant (all *F*s < 2.22, all *p*s > .12, all η_p^2 < .06).

Extinction. During extinction, the main effects of type of CS and all the interactions with type of CS were not significant (all *F*s < .61, all *p*s > .60, all η_p^2 < .03).

3.1.2 Self-report reactions to the conditioned stimulus

Preconditioning. The main effects of type of CS and type of CS by subject group interaction were not significant for all self-report measures (all Fs < .94, all *p*s > .40, all η_p^2 < .04).

Conditioning. We found evidence of conditioning at the subjective level for the whole sample, as revealed by a significant main effects of type of CS for the self-report measures of anxiety ($F(2, 84) = 8.82$, $p < .001$, $\eta_p^2 = .17$), unpleasantness ($F(2, 71.35) =$

12.89, $p < .001$, $\eta_p^2 = .24$) and arousal ($F(2, 84) = 15.29$, $p = < .001$, $\eta_p^2 = .27$). There were linear increases from CS_{pos} to CS_{neu} to CS_{neg} for anxiety ($F(1, 42) = 9.87$, $p = .003$, $\eta_p^2 = .19$) and unpleasantness ($F(1, 42) = 14.55$, $p < .001$, $\eta_p^2 = .26$) and there was a quadratic trend for arousal ($F(1, 42) = 28.50$, $p < .001$, $\eta_p^2 = .40$; see Figure 2). However, conditioning did not differ between groups, as indicated by the non-significant interactions between type of CS and subject group for any of these variables (all Fs < 1.60, all ps > .18, all $\eta_p^2 < .07$). A posthoc test comparing the CS_{neg} vs. CS_{pos} scores for each subjective variable (anxiety, unpleasantness, and arousal) confirmed the absence of differences in conditioning across groups (all Fs < 1.19, all ps > .31, all $\eta_p^2 < .05$).

Extinction. During extinction, and similar to conditioning, main effects of type of CS were found for anxiety, unpleasantness, and arousal (all Fs > 9.31, all ps < .001, all $\eta_p^2 > .18$), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for anxiety ($F(1, 42) = 9.10$, $p = .004$, $\eta_p^2 = .18$) and unpleasantness ($F(1, 42) = 18.68$, $p < .001$, $\eta_p^2 > .31$), and a quadratic trend for arousal ($F(1, 42) = 24.97$, $p < .001$, $\eta_p^2 = .37$). The type of CS by subject group interactions with anxiety and unpleasantness were not significant (both Fs < 1.65, ps > .17, $\eta_p^2 = .07$) but significance was approached for the arousal ratings ($F(4, 84) = 2.28$, $p = .07$, $\eta_p^2 = .10$). Pair-wise comparisons revealed that the arousal ratings for the CS_{pos} were significantly higher for SAD ($t(47) = 2.44$, $p = .05$, $d = .35$) and PDA patients ($t(47) = 2.61$, $p = .04$, $d = .37$) in comparison to HC.

3.1.3 Self-report reactions to the unconditioned stimulus

Main effects of type of US were found for the self-report measures of happiness ($F(2, 63.09) = 50.67$, $p < .001$, $\eta_p^2 = .55$) and anxiety ($F(2, 73.35) = 20.53$, $p < .001$, η_p^2

= .33), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for the anxiety ratings ($F(1, 42) = 23.84$, $p < .001$, $\eta_p^2 = .36$) and linear decreases from CS_{pos} to CS_{neu} to CS_{neg} for the happiness ratings ($F(1, 42) > 66.79$, $p < .001$, $\eta_p^2 = .61$). However, there were no differences in the happiness or anxiety ratings of the US between groups, as shown by the non-significant type of US by group interactions (both Fs < .89, both ps > .47, all $\eta_p^2 <$ .04).

3.1.4 Comorbidity, medication, and SAD subtype

We repeated the above analyses but excluded the three participants from the SAD group who had comorbid Generalized Anxiety Disorder (GAD), the participants in the PDA group who were taking medication and the four SAD participants who did not meet the criteria for generalized SAD. None of these analyses had any significant effect on the results (data not shown).

3.2 Study 2

The demographic and psychometric characteristics of the participants in Study 2 are reported in Table 2.

3.2.1 Startle EMG

Preconditioning. The main effects of type of CS, type of CS by subject group and type of CS by time interactions were not significant (all Fs < 2.13, all ps > .13, all η_p^2 < .06).

Conditioning. Although a type of CS by time interaction emerged ($F(6, 172.2) = 3.57$, $p < .005$, $\eta_p^2 = .10$) and follow-up analyses revealed significant differences between the CS_{neg} and CS_{pos} ($t(39) = 3.56$, $p < .005$, $d = .56$) for the whole sample, these interactions were in the opposite direction than we predicted, with higher responses to the CS_{pos} than to the CS_{neg} in the fourth block of the conditioning phase. In any case, the main effects of type of CS and the interactions between type of CS and subject group and between type of CS by time and subject group were not significant (all Fs < 1.58, all ps > .15, all $\eta_p^2 < .04$; Figure 3).

Reminder. As in Study 1, during the reminder phase, the main effect of type of CS and all the interactions with type of CS were not significant (all Fs < 1.14, all ps > .33, all $\eta_p^2 < .03$).

Extinction. The main effects of type of CS and all the interactions including type of CS were not significant (all Fs < 1.44, all ps > .21, all $\eta_p^2 < .04$).

3.2.2 Self-report reactions to the conditioned stimulus

Preconditioning. As in Study 1, the main effect of type of CS and type of CS by subject group interaction were not significant for all self-report measures (all Fs < .1.34, all ps >.25, all $\eta_p^2 < .04$).

Conditioning. As in Study 1, main effect of type of CS were found for the self-report measures of anxiety ($F(2, 72) = 28.27$, $p < .001$, $\eta_p^2 = .44$), unpleasantness ($F(2, 72) = 29.78$, $p < .001$, $\eta_p^2 = .45$) and arousal ($F(2, 58.59) = 23.97$, $p = < .001$, $\eta_p^2 = .40$), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for anxiety ($F(1, 36) = 40.16$, $p < .001$, $\eta_p^2 = .53$) and unpleasantness ($F(1, 36) > 49.29$, $p < .001$, $\eta_p^2 > .58$), and a quadratic

trend for arousal ($F(1, 36) = 33.40$, $p < .001$, $\eta_p^2 = .48$; see Figure 4). However, as in Study 1, the type of CS by subject group interactions for anxiety, unpleasantness and arousal were not significant (all Fs < 1.92, all ps > .16, all $\eta_p^2 < .05$). Again, a posthoc test comparing the CS_{neg} vs. CS_{pos} scores for each subjective variable (anxiety, unpleasantness, and arousal) revealed a significant difference only for the arousal ratings ($F(1, 36) = 5.63$, $p < .02$, $\eta_p^2 = .14$). Pair-wise comparisons indicated higher arousal ratings for the CS_{neg} vs. CS_{pos} in the SSA group ($t(39) = 3.09$, $p = .01$, $d = .49$) but not in the HC group ($t(39) = .28$, $p = 1.00$, $d = .04$).

Extinction. Main effects of type of CS were found for the anxiety ($F(2, 72) = 18.44$, $p < .001$, $\eta_p^2 = .34$), unpleasantness ($F(2, 72) = 20.36$, $p < .001$, $\eta_p^2 = .36$), and arousal ratings ($F(2, 72) = 13.43$, $p < .001$, $\eta_p^2 = .27$), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for anxiety ($F(1, 36) = 19.75$, $p < .001$, $\eta_p^2 = .35$) and unpleasantness ($F(1, 36) = 27.10$, $p < .001$, $\eta_p^2 = .43$), and a quadratic trend for arousal ($F(1, 36) = 17.98$, $p < .001$, $\eta_p^2 = .33$). As in Study 1, the type of CS by subject group interactions for anxiety, unpleasantness and arousal were all not significant (all Fs < .84, all ps > .44, all $\eta_p^2 < .02$).

3.2.3 Self-report reactions to the unconditioned stimulus

Main effects of type of US were found for the self-report measures of happiness ($F(2, 57.41) = 56.41$, $p < .001$, $\eta_p^2 = .61$) and anxiety ($F(2, 72) = 23.33$, $p < .001$, $\eta_p^2 = .39$), with linear increases from CS_{pos} to CS_{neu} to CS_{neg} for the anxiety ratings ($F(1, 36) = 34.02$, $p < .001$, $\eta_p^2 = .49$) and linear decreases from CS_{pos} to CS_{neu} to CS_{neg} for the happiness ratings ($F(1, 36) = 75.12$, $p < .001$, $\eta_p^2 = .68$). As in Study 1, there were no

between groups differences in the happiness or anxiety ratings for the US, as shown by the non-significant type of US by group interactions (both Fs < .1.69, both ps > .19, all $\eta_p^2 < .05$).

4. Discussion

We employed a novel social conditioning paradigm using socially relevant US to study fear conditioning in individuals with social anxiety in two separate studies. In both studies, and for all groups, we found evidence of fear conditioning for the subjective but not the physiological (i.e., FPS) measures. Contrary to our hypotheses, we found no evidence of enhanced fear conditioning (in either subjective or physiological measures) in socially anxious individuals (clinical or sub-clinical) in comparison to healthy controls or to another clinical group (PDA patients).

In human fear conditioning experiments, it is relatively common for conditioning responses to differ across response systems (see Beckers et al., 2012), as shown here. In the present study, we used FPS as a physiological index of conditioning, and, according to Lipp, Sheridan & Siddle (1994), the startle reflex can be potentiated as long as the eliciting stimuli is sufficiently aversive. This may suggest that the US employed here were not experienced as aversive enough to elicit FPS. However, our anxiety ratings to the negative US were in fact higher than those of Lissek et al. (2008).

The fact that in our two studies the SAD (or SSA) individuals did not show enhanced fear conditioning compared to healthy controls is at odds with the results from Lissek et al. (2008) and is surprising given that the acquisition phase was identical across our two studies and the study by Lissek and colleagues. Furthermore, our samples of

socially anxious participants (both clinical and subclinical) presented similar (or higher) social anxiety severity to those of Lissek et al. and our control individuals also had similar or lower social anxiety severity as those of Lissek et al. (2008). Although we included some individuals with non-generalized SAD in our study, excluding them did not change the results. In any case, it must be noted that there is little evidence to support the subtyping of SAD (see Stein et al., 2010 or Aderka, Nickerson & Hoffman, 2012).

There are, however, some differences between our socially anxious samples and those of Lissek in terms of gender distribution, comorbidity, and ethnicity. The majority (75-80%) of our participants were women, whereas in Lissek's study, the sample was more evenly distributed (55% female). Although, as in Lissek et al. (2008), we did not find a main effect of gender in our analyses, growing evidence suggests that men and women may diverge in their acquisition of fear (Inslicht et al., 2012). These recent data and the fact that three previous studies on fear conditioning and social anxiety with negative results examined only males (Schneider et al., 1999; Hermann et al., 2002; Veit et al., 2002), whereas three studies with positive results included both males and females (Lissek et al., 2008, Pejic et al., 2011, Davis et al., 2010), indicate that gender should be further studied as a possible confounding/explanatory variable in fear acquisition, especially in clinical samples.

With regards to comorbidity, almost 80% of SAD patients in Lissek et al. (2008) had a current or past comorbid mental disorder, whereas in our study of SAD patients only 19% presented with a comorbid GAD. This raises the possibility that differences between our FPS results and the results of Lissek et al. (2008) are partially explained by the presence of comorbidity. However, the results of a series of recent studies using

aversive imagery in anxiety disorders (McTeague & Lang, 2012) suggest that comorbidity typically *decreases* FPS and therefore in our "purer" SAD sample (and also in our SSA sample) the differences in our physiological measures should have been more evident.

In relation to ethnicity, approximately half of Lissek's participants were Caucasian, whereas our entire sample population was Caucasian. Some recent data suggest that fear conditioning may be facilitated and fear extinction may be impaired if CS of another ethnic group are used (Olsson, Ebert, Banaji & Phelps, 2005). Therefore, one possible explanation for the discrepant findings between our study and Lissek et al. (2008) is that the findings in the latter study, at least in part, reflect a general startle reflex potentiation effect because of the CS stimuli used.

It is noteworthy that at least four studies (Schneider et al., 1999; Hermann et al., 2002; Veit et al., 2002), including the present study, have not detected enhanced "conditionability" in SAD individuals in comparison to healthy controls. Although previous research on anxiety disorders seems to support the idea that enhanced conditionability is a characteristic of PTSD (e.g., Orr et al., 2000), results regarding other anxiety disorders are mixed. For example, at least two previous studies did not find evidence of enhanced conditionability in panic disorder (Michael et al., 2007, Del Ben et al., 2001) or GAD (Pitman & Orr, 1986), but a recent study found enhanced conditioning in individuals with specific phobias (Vriends, Michael, Schindler & Margraf, 2011). In any case, methodological differences between studies make it difficult to come to conclusions in this area (see Lissek et al., 2005).

Although, as stated in the introduction, a history of traumatic experiences is common among individuals with SAD, other mechanisms, as opposed to increased fear acquisition, could be "abnormal" in SAD. These include other processes related to fear learning, such as impaired extinction, as well as non-associative processes.

Impaired fear extinction seems to characterize individuals with certain anxiety disorders, including PTSD (Blechert et al., 2007; Orr et al., 2000; Peri, Ben-Shakhar, Orr & Shalev, 2000) and PD (Michael et al, 2007). One possibility is that SAD is characterized by a deficit in fear extinction rather than in fear acquisition. To date, only one study has reported that SAD patients show impaired (delayed) extinction (Hermann et al., 2002). In our two studies, we did not find differences in fear extinction between socially anxious individuals (clinical or subclinical) and healthy controls. However, the fact that, as in previous studies (Schneider et al., 1999; Hermann et al., 2002; Veit et al., 2002), we found no evidence of fear acquisition limits the interpretation of our extinction data. In any case, in the only study where SAD individuals showed enhanced fear acquisition (Lissek et al., 2008); there were no between-group differences in extinction. We must also note that our extinction phase was relatively short. Future applications of the paradigm employed here should include a larger extinction phase.

Finally, accounts of SAD that are not based on fear conditioning mechanisms exist. An important body of research suggests that cognitive and attentional processes play a major role in the origins or maintenance of pathological social anxiety. For example, cognitive-behavioral models of SAD suggest that distortions in the processing of social information lead to heightened anxiety in social situations which, in turn, helps to maintain social anxiety (Rapee & Heimberg, 1997; Clark & Wells; 1995). Other

theoretical models focus on the attentional bias for threat that seems to characterize SAD patients (Mogg, Phillpot & Bradley, 2004). The results of the present study suggest that these alternative explanations may have greater weight for understanding social anxiety.

The present study has several limitations. First, our sample sizes were relatively small, albeit similar to previous studies in the field. Second, our participants in the PDA group could be using SSRIs, but previous data suggest that this class of medication does not affect the FPS (Grillon, Chavis, Covington & Pine, 2009). Finally, as noted above, our paradigm was not specially suited to the study of extinction processes.

5. Conclusions

Social anxiety does not seem to be related to "abnormal" fear conditioning. Other associative or non-associative processes seem to play a greater role in explaining social anxiety.

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TABLE 1. Demographic and clinical characteristics of the participants in Study 1.

	Group[a]								
	Social Anxiety Disorder (*n*=16)		Panic Disorder *(n*=16)		Healthy Controls *(n*=16)				
Variable	Mean	SD	Mean	SD	Mean	SD	F	df	P[d]
Age	25.81	8.50	33.00	8.65	31.06	10.66	2.54	2, 47	ns
LSAS total score (0-144)	76.56_a	22.72	40.13_b	23.29	17.88_c	7.70	37.69	2, 47	<.0001
Anxiety/fear	41.63_a	10.35	20.75_b	11.75	10.50_c	5.41	43.98	2, 47	<.0001
Avoidance	34.94_a	13.16	19.38_b	13.35	7.38_c	3.72	25.11	2, 47	<.0001
FNES (0-30)	25.31_a	4.92	18.31_b	5.64	8.75_c	4.58	43.06	2, 47	<.0001
BDI (0-63)	19.25_a	9.57	16.56_a	9.22	2.01_c	2.51	22.41	2, 47	<.0001
STAI-Trait (0-60)	36.00_a	10.62	29.79_a	10.71	10.81_c	5.94	29.91	2, 43	<.0001
PDSS-SR (0-28)	-	-	12.63	5.89	-	-			
Airpuff rating (0-10)	7.87	4.13	6.25	1.34	6.88	.81	1.65	2, 47	ns
	N	%	N	%	N	%	χ^2	df	
Female gender	13	81.3	11	68.8	13	81.3	0.943	2	ns
Psychiatric comorbidity									
GAD	3	19	0	0	0	0			
Current medication (SSRIs)	0	0	10	62.5	0	0			

LSAS, Liebowitz Social Anxiety Scale; FNES, Fear of Negative Evaluation Scale; BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; PDSS-SR, Panic Disorder Severity Scale self-report; GAD, Generalized Anxiety Disorder; SSRIs, Selective Serotonin Reuptake Inhibitors.

[a]Means with different subscripts differ significantly at p<0.05.

[d]One-way ANOVA (except for gender, which was assessed with a Chi square)

TABLE 2. Demographic and psychometric characteristics of the participants in Study 2.

	Sub-clinical Social Anxiety (*n*=20)		Healthy Controls (*n*=20)				
Variable	Mean	SD	Mean	SD	F	df	P[a]
Age	20.15	2.23	21.55	2.60	16.92	1, 39	ns
LSAS total score (0-144)	67.80	13.49	17.15	6.61	227.25	1, 39	<.0001
Anxiety/fear	35.70	7.71	8.85	3.75	196.41	1, 39	<.0001
Avoidance	32.10	7.63	8.30	4.52	144.07	1, 39	<.0001
FNES (0-30)	20.25	6.18	7.95	5.12	46.87	1, 39	<.0001
BDI (0-63)	11.47	9.16	4.50	4.80	9.00	1, 39	P=.005
STAI-Trait (0-60)	23.63	10.28	10.65	4.62	24.60	1, 39	<.0001
Airpuff rating (0-10)	6.45	1.50	4.65	2.56	7.35	1, 39	P=.010
	N	%	N	%	χ^2	df	
Female gender	15	75	15	75	0.00	1	ns

LSAS, Liebowitz Social Anxiety Scale; FNES, Fear of Negative Evaluation Scale; BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; PDSS-SR, Panic Disorder Severity Scale self-report.

[a]Two tailed t-tests (except for gender, which was assessed with a Chi square)

FIGURE 1. Average, Standardized Startle-Blink EMG Magnitudes During Preconditioning, Conditioning, and Reminder phases (Study 1)

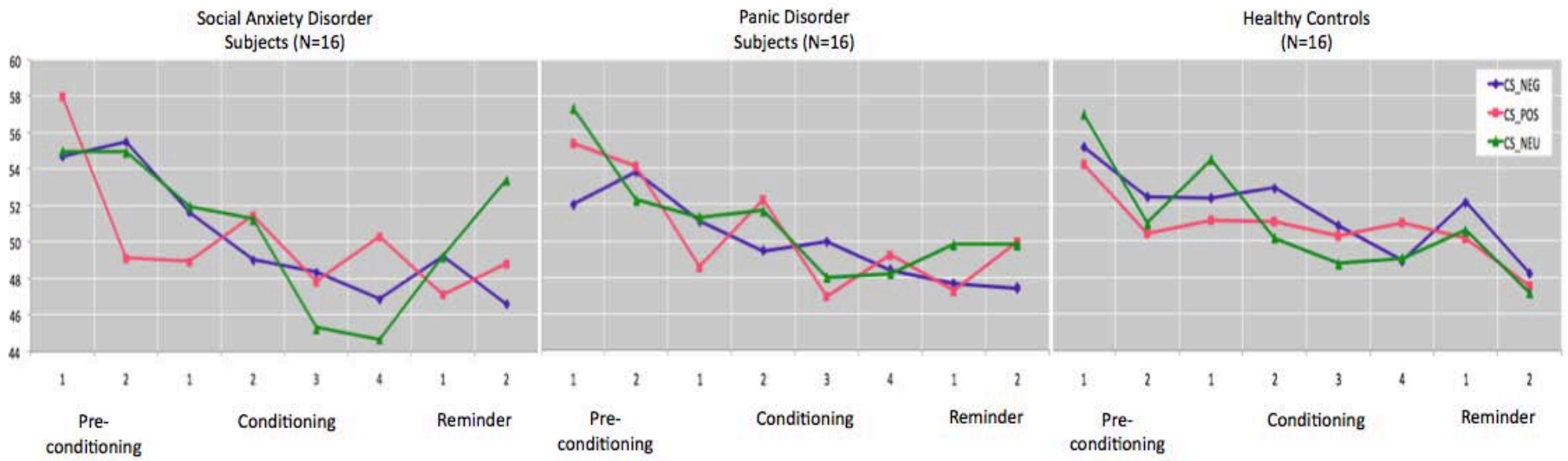

Figure 2. Likert Scale Scores for Self-Report Measures of Anxiety, Unpleasantness, and Arousal During Conditioning (Study 1).

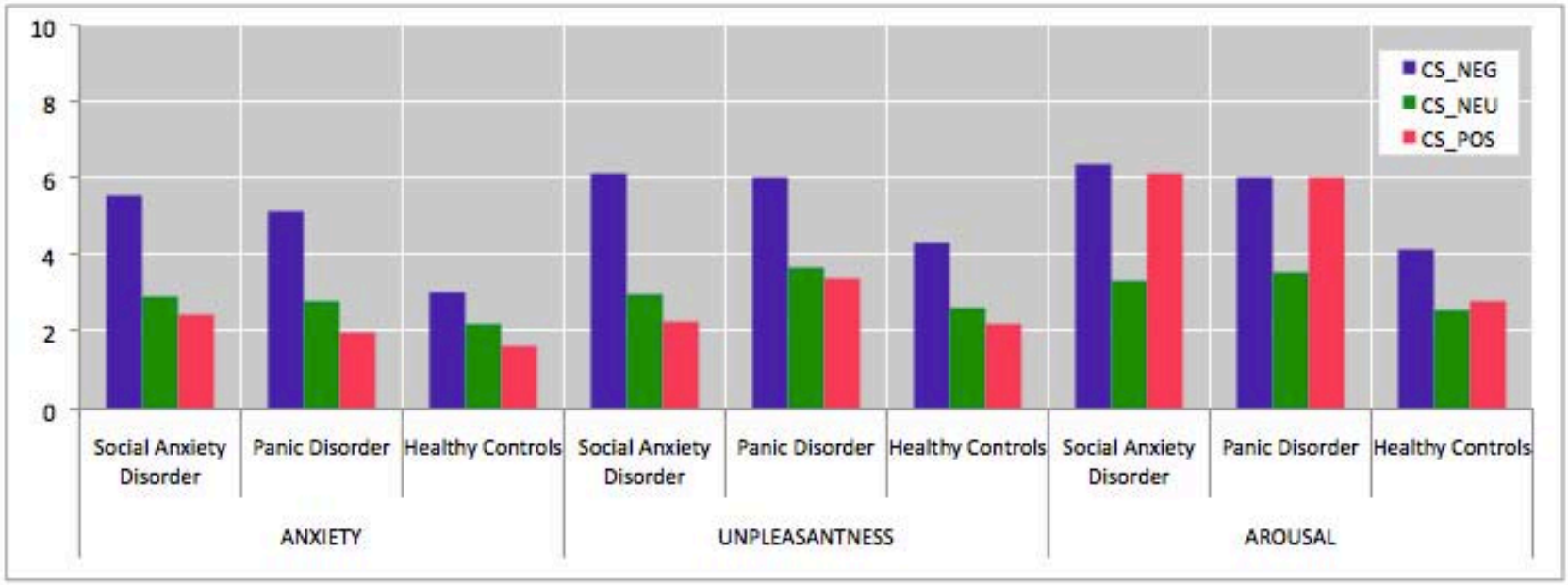

Figure 4. Likert Scale Scores for Self-Report Measures of Anxiety, Unpleasantness, and Arousal During Conditioning (Study 2).

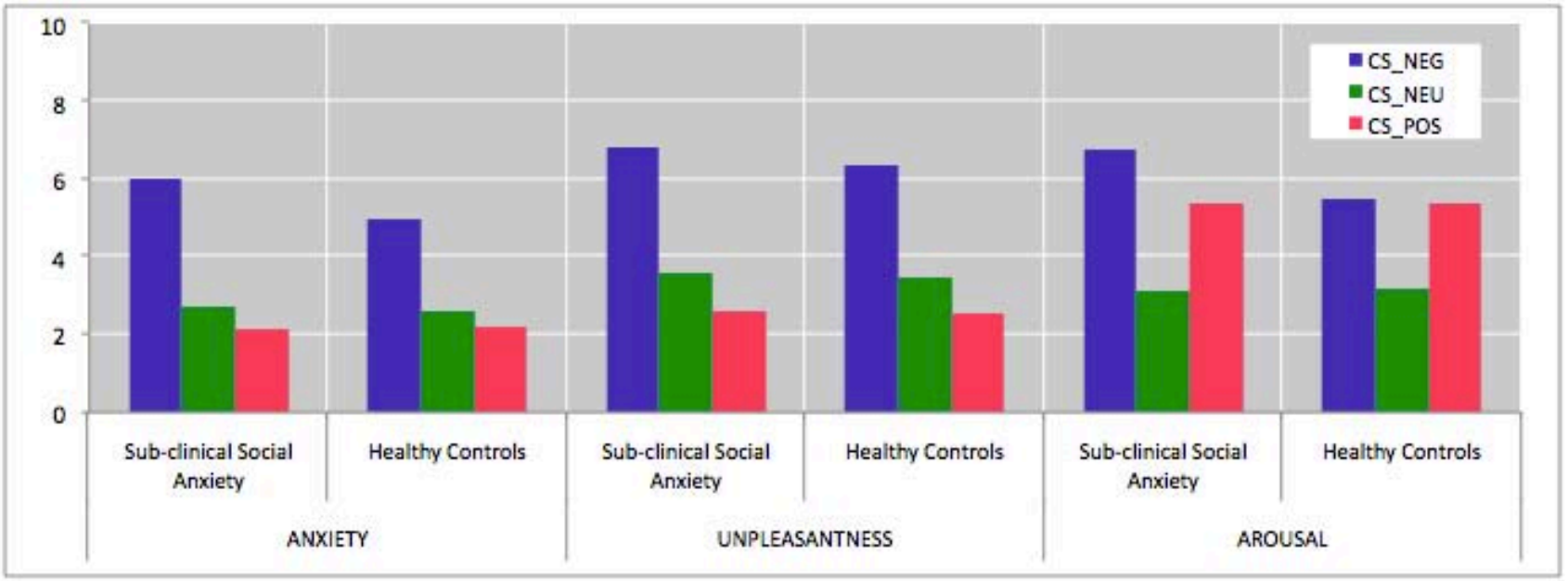

FIGURE 3. Average, Standardized Startle-Blink EMG Magnitudes During Preconditioning, Conditioning, and Reminder phases (Study 2)