To approach or to avoid : neurobiological mechanisms in social anxiety

Peer, J.M. van

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Jacobien Marit van Peer

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	Dr. M. Rinck, Radboud Universiteit Nijmegen

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Chapter 1

General Introduction

Extensive animal research suggests that the Hypothalamus-Pituitary-Adrenal (HPA) system and the associated release of glucocorticoids (cortisol in humans) play an important role in the regulation of social motivational behavior. For example, in nonhuman primates elevated cortisol levels have been related to increased social submissiveness, fearful temperament and avoidance behavior in social situations (Kalin, Larson, Shelton, & Davidson, 1998; Sapolsky, 1990). Also in humans, the relation between HPA-function and withdrawal behaviors, particularly behavioral inhibition, has received a great deal of attention in the developmental literature (e.g., Kagan, Reznick, & Snidman, 1987; Schmidt et al., 1997; Spangler & Schieche, 1998). Such a relationship between HPA-function and social behavior is of particular relevance to patients with social anxiety disorder (SAD), which is characterized by extreme fear and avoidance of social situations, and for which childhood inhibition has been identified as a risk factor (for reviews see e.g., Hirshfeld-Becker, Micco, Simoes, & Henin, 2008; Rubin, Coplan, & Bowker, 2009). However, thus far little is known about the causal role of cortisol in the regulation of human social fear and avoidance behavior. The aim of this thesis is to gain more insight in the psychobiological mechanisms underlying social fear and avoidance behavior in humans, in particular socially anxious individuals, and the role of cortisol in the regulation of these processes.

In this introduction, I will start with a description of the main characteristics of social anxiety, and current knowledge about the role of information processing biases in this disorder. The next paragraph explains how these processes may be linked to behavior, including a discussion of the brain mechanisms involved in the regulation of motivational behavior and the role of individual differences. The second main part of this introduction focuses on the role of cortisol in the regulation of social motivational behavior. The introduction ends with an overview of the main predictions, the overall experimental approach, and an outline of the studies described in each of the remaining chapters of this thesis.

Fear and avoidance in social anxiety

Social anxiety disorder (SAD) is the most common anxiety disorder, with lifetime prevalence rates ranging from 7 to 13% in Western countries (see Furmark, 2002, for a recent review). SAD is characterized by extreme fear and avoidance of social situations (American Psychiatric Association [APA], 1994). Central to this disorder is the fear of behaving embarrassingly and being evaluated negatively by others. Cognitive behavioral models of social anxiety emphasize the role of information processing biases and avoidance or safety behaviors in the etiology and maintenance of this disorder. Two influential models (Clark & Wells, 1995; Rapee & Heimberg, 1997) both emphasize increased attention to threat as a critical factor in the maintenance of social fear. According to these models, socially anxious individuals are characterized by strong self-focused attention towards internal threat cues, such as dysfunctional assumptions about social evaluation and symptoms of physiological arousal (see Clark & Wells, 1995). In addition, Rapee and Heimberg (1997) suggested they show heightened vigilance to *environmental* cues related to potential negative evaluation, i.e., social threat.

Such preferential processing of external threat has been investigated in a wide range of cognitive-experimental studies, mainly through measurement of reaction times in response to threatening versus neutral stimuli in emotional Stroop, dot probe, or emotional spatial cueing tasks (see e.g., Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & Van IJzendoorn, 2007; Mobini & Grant, 2007, for recent reviews). The most widely used social threat stimuli in these tasks have been words (e.g., 'criticize') or pictures of human faces (e.g., angry compared to neutral or happy expressions). Results of these studies reliably demonstrated the existence of a threat-related attentional bias, in both clinical samples and individuals with high self-reported levels of social anxiety (see Bar-Haim et al., 2007). In addition, functional neuroimaging studies have shown hyperactive amygdala responses to threatening faces in patients with SAD (Phan, Fitzgerald, Nathan, & Tancer, 2006; Stein, Goldin, Sareen, Zorrilla, & Brown, 2002; Straube, Kolassa, Glauer, Mentzel, & Miltner, 2004). Thus, it seems that social anxiety is characterized by a hyperresponsive alert system, with an attentional bias towards socially threatening stimuli. In addition, some behavioral studies have provided evidence suggesting that this initial vigilance is followed by an avoidance of threat cues

in later, more strategic processing stages (e.g., Amir, Foa, & Coles, 1998; Mogg, Philippot, & Bradley, 2004; see also Mogg, Bradley, DeBono, & Painter, 1997).

But what about behavior? In addition to fear and sensitivity to social threat, a second main characteristic of SAD is avoidance of social situations. Because such avoidance behavior reduces the opportunity to habituate to or reappraise a feared situation, or to learn to cope with the anxiety, it is considered to be a major maintaining factor of anxiety symptoms in the long-term (e.g., Clark & Wells, 1995). In contrast to attentional processes, however, avoidance behavior has not been a major focus of experimental research. The present thesis aims to start filling in this gap, with a main focus on overt social avoidance behavior.

Motivational systems regulating approach and avoidance behavior

Many authors have emphasized a close relationship between affective evaluations and action tendencies (e.g., Chen & Bargh, 1999; Frijda, Kuipers, & Ter Schure, 1989; Lang, Bradley, & Cuthbert, 1990, 1992). According to these views, positive and negative emotions and evaluations are strongly linked to approach and avoidance behavior, respectively, and this association is mediated by distinct appetitive and aversive motivational systems in the brain. For example, Gray (e.g., 1987; Gray & McNaughton, 2000) proposed a behavioral activation system (BAS) which responds to incentives, regulates movements towards goals, and is associated with the experience of positive affect. On the other hand, a behavioral inhibition system (BIS)[1] responds to threat, resulting in the inhibition of behavior (or avoidance) and is associated with the experience of negative affect (see e.g., Carver, Sutton, & Scheier, 2000, for an overview of similar theories).

Importantly, it has been suggested that individuals differ in the relative sensitivity or activation of these motivational systems, resulting in a predisposition to engage in either approach or avoidance behavior, and a proneness to react to reward or threat, and to experience positive or negative affect. To assess these individual differences, Carver and White (1994) created self-report scales (the BIS-BAS scales),

[1] Note that in a recent revision of this theory (Gray & McNaughton, 2000) the regulation of responses to aversive stimuli is now ascribed to the Fight-Flight-and Freezing system instead of the BIS, whereas the BIS is a conflict detection and resolution device that inhibits ongoing behavior (see also Smillie Pickering, & Jackson, 2006).

which have been widely used in experimental research. Extreme activation or de-activation of either of these systems has also been related to vulnerability for psychopathology. For example, hypo-activation in the approach system has been associated with depression (see e.g., Davidson, 1998), whereas hyperactivity of the behavioral inhibition system has been associated with anxiety (e.g., Gray & McNaughton, 2000). In this thesis, I will investigate avoidance behavior in both healthy individuals with high versus low self-reported levels of behavioral inhibition (BIS) and patients with SAD.

Brain mechanisms underlying emotion processing and motivational behavior

The processing of emotional stimuli and the regulation of the associated approach and avoidance responses involves a complex circuitry involving various cortical and subcortical brain regions. A first important structure in this network is the amygdala (for a review see e.g., Phelps, 2006) which receives input about the emotional significance of a stimulus quickly and prior to awareness (e.g., Morris, Öhman, & Dolan, 1998; Whalen et al., 1998). It has been suggested that projections from the amygdala to sensory cortical regions (Amaral, Behniea, & Kelly, 2003; Anderson & Phelps, 2001; Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004) are able to facilitate further attentional and perceptual processes, resulting in increased cortical attention and vigilance in situations of danger (e.g., Whalen, 1998). Furthermore, direct and indirect output connections from the amygdala activate motivational systems, which enable goal-directed approach and avoidance reactions to emotional stimuli. The prefrontal cortex (PFC), in particular the anterior cingulate (ACC) and orbitofrontal (OFC) regions, plays an important role in these motivational systems (see e.g., Blair & Cipolotti, 2000; Hornak et al., 2003; Kringelbach & Rolls, 2003; LeDoux, 2002; Roelofs, Minelli, Mars, Van Peer, & Toni, 2009; Rolls, 2000). Davidson and colleagues (see e.g., Davidson, 2004) proposed that specialized neural substrates for behavioral approach and withdrawal systems are lateralized in the left and right prefrontal cortex, respectively. Support for this notion comes from EEG studies showing a relation between baseline measures of prefrontal activation asymmetry and individual differences in dispositional mood, affective reactivity, and temperament. In these studies, relative left-sided prefrontal activation has been associated with more positive affect, increased reactivity to positive stimuli, and relatively higher levels of self-reported behavioral activation (BAS),

whereas more relative right frontal activation has been related to more negative affect, increased reactivity to negative stimuli, and relatively higher levels of behavioral inhibition (BIS) (see e.g., Davidson, 1998; Sutton & Davidson, 1997; Tomarken, Davidson, Wheeler, & Doss, 1992; Wheeler, Davidson, & Tomarken,1993) .

Interestingly, in primates many of the areas involved in this emotional-motivational network are highly sensitive to emotional facial stimuli, which underscores the important role of these networks in social interaction (see e.g., Rolls, 2000). Functional neuroimaging studies in humans have shown that viewing angry or fearful faces activates the ACC, OFC, and amygdala in particular (for an overview see Adolphs, 2002; McClure et al., 2004; Strauss et al., 2005). Furthermore, several of these areas have been shown to be hyperresponsive to threatening emotional expressions in socially anxious individuals compared to healthy controls (e.g., Phan et al., 2006; Stein et al., 2002; Straube et al., 2004). In addition, increased subcortical and decreased cortical activity have been found in SAD during (anticipation of) public speech (Lorberbaum et al., 2004; Tillfors et al., 2001), which is consistent with the notion that one function of the PFC is to modulate or inhibit amygdala activity (for a review see e.g., Phelps, 2006), and suggests a failure in prefrontal inhibition of amygdala driven fear responses in SAD.

In the next section, I will describe how the stress hormone cortisol may affect this network and, consequently the processing and regulation of emotions and motivational behavior.

Hormones and behavior: role of the HPA-axis and cortisol

Role of the HPA-axis in response to stress

The HPA-axis consists of the hypothalamus, the pituitary gland, and the adrenal gland. Together with the sympathetic nervous system, this system plays a primary role in the stress-response (see e.g., De Kloet, Joëls, & Holsboer, 2005; Sapolsky, Romero, & Munck, 2000, for reviews). In reaction to the perception of a stressor, the hypothalamus releases cortisol-releasing factor (CRF), which triggers the release of ACTH in the pituitary. This, in turn, causes adrenal secretion of glucocorticoids (GC, cortisol in humans). Finally, negative feedback mechanisms cause elevated GC concentrations to inhibit subsequent HPA activity, to prevent the stress-response from overshooting. GC are important for the regulation of adaptive stress responses. For example, they increase

activity of the sympathetic nervous system and enhance the mobilization of energy sources that are needed for action (e.g., fight or flight). In contrast, they inhibit parasympathetic functions that are unnecessary in the context of immediate threat, such as growth, reproduction, and inflammation. In addition to these actions during acute stress, both basal and stress-induced GC serve preparative functions to prime the defense mechanisms for responses to future stressors (Sapolsky et al., 2000).

Glucocorticoids can easily cross the blood–brain barrier (e.g., Herbert et al., 2006) and access the brain where they bind to receptors. There are two types of GC receptors in the brain: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) (e.g., De Kloet, 1991; De Kloet, Oitzl, & Joëls, 1999). GCs have higher affinity (i.e., bind more readily) to MRs than to GRs, resulting in a predominant occupation of MRs when GC levels are in basal ranges, whereas GRs are occupied only at the peak of the circadian cycle or when cortisol levels are elevated due to stress (e.g., De Kloet et al., 1999) or exogenous administration of cortisol. These receptors are also differentially distributed in the brain. The MR receptor is exclusively present in the limbic system, whereas the GR receptor is present in both subcortical and cortical structures, with a preferential distribution in the prefrontal cortex. GC effects on cognitive function are mediated by the relative activation of MR and GR receptors (De Kloet, 1991; De Kloet et al., 1999; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007).

Cortisol and social motivation

Animal studies suggest that GC play an important role in the regulation of social motivational behavior. For example, studies in nonhuman primates have shown that elevated GC levels are related to the manifestation of increased submissiveness and avoidance behavior in social situations (Sapolsky, 1990). There are also some indications that patients with SAD have increased cortisol stress responses (Condren, O'Neill, Ryan, Barrett, & Thakore, 2002; Furlan, DeMartinis, Schweizer, Rickels, & Lucki, 2001), although this was not confirmed in other studies (Levin et al., 1993; Martel et al., 1999). In addition, relatively increased right PFC activity, which is related to fearful temperament and behavioral inhibition, has been associated with higher cortisol levels in rhesus monkeys (Kalin et al., 1998a; Kalin, Shelton, & Davidson, 2000) and human infants (Buss et al., 2003). The PFC is an important target structure for GC (e.g., Meaney & Aitken, 1985; Radley et al., 2004), and exogenously administered cortisol has been

shown to affect prefrontal functions such as working memory in humans (for reviews see Wolf, 2003; Lupien et al., 2007). Together, the relation between HPA-axis function and social behavior on the one hand, and the effects of cortisol on prefrontal brain areas involved in the regulation of social behavior on the other hand, give rise to the hypothesis that cortisol plays an important role in the prefrontal regulation of social fear behavior.

To summarize, cortisol and avoidance behavior may play an important role in social anxiety. However, experimental studies in social anxiety have predominantly focused on emotion processing and attention, and studies investigating avoidance behavior are largely lacking. Furthermore, little is known about the effects of cortisol on prefrontal regulation of avoidance behavior in humans, or even about effects of cortisol on cognitive and emotional processes other than memory (see Lupien et al., 2007, for a review), such as attentional processing of threat. Two recent studies using an emotional Stroop task indicated that increased basal cortisol levels (e.g., Van Honk et al., 1998), and high cortisol levels due to cortisol administration (Putman, Hermans, Koppeschaar, Van Schijndel, & Van Honk, 2007) were associated with relative attentional avoidance of threat. However, the effect of cortisol on *overt* avoidance *behavior* in humans remains unexplored. It is relevant to gain more insight in these mechanisms, not only to increase our understanding of the role of HPA-axis dysfunctions in the etiology and maintenance of social anxiety, but also because the administration of cortisol has recently been proposed as a treatment for SAD (Soravia et al., 2006).

Outline of this thesis

Main aim, predictions and general methodology

The aim of this thesis is twofold: First, I want to gain more insight in the brain processes underlying threat processing and avoidance behavior, especially in high socially anxious individuals. Second, I will investigate how these processes are affected by cortisol. The following hypotheses will be tested:

1. Threatening stimuli, in particular angry faces, receive preferential processing by high socially anxious individuals, and such vigilance occurs in early processing stages.
2. Individuals characterized by high levels of behavioral inhibition or social anxiety show stronger avoidance tendencies in reaction to social threat.
3. Threat processing and avoidance are facilitated by high levels of endogenous or exogenous cortisol.

Overall, these predictions will be investigated using the following methods:

First, a computerized reaction time (RT) paradigm is applied to measure approach and avoidance responses in reaction to social stimuli. In this task (the approach-avoidance (AA)-task, e.g., Rotteveel & Phaf, 2004), participants evaluate the emotional expression of photographs of happy and angry faces by making an approaching (flexion) or avoiding (extension) arm movement. The AA-task consists of an affect-congruent condition, involving approach movements to happy faces and avoidance movements to angry faces and an affect-incongruent condition in which the instruction is reversed. Typically, reaction times are faster in the affect-congruent than the affect-incongruent condition, reflecting the general tendency of participants to approach pleasant and avoid unpleasant stimuli (see e.g., Chen & Bargh, 1999; Rinck & Becker, 2007; Roelofs, Elzinga, & Rotteveel, 2005; Rotteveel & Phaf, 2004; Solarz, 1960). Although such a computer task constitutes an artificial and highly simplified environment compared to 'real-life' social interactions, it ensures a direct and objective measure of behavior, and also makes it possible to measure brain activity during task performance.

Second, brain activity (in the form of event-related potentials) is recorded from the scalp during task performance to get more insight in the neural processes associated

with threat processing and avoidance behavior. Event-related potentials (ERPs) provide a continuous and high temporal resolution measure of both the speed (latency) and intensity (amplitude) of cerebral processing and are therefore very suitable for a refined investigation of biases in different information processing stages. Resting state EEG is also measured, to investigate individual differences in (and cortisol effects on) baseline motivational brain states.

Third, effects of cortisol on threat processing and behavior are investigated through experimental manipulation of endogenous cortisol levels (with a psychosocial stress task) as well as through acute administration of exogenous cortisol. Because many factors interact with endogenous cortisol levels during stress-induction (e.g., arousal, social stress context, and individual differences), the emphasis in this thesis lays on exogenous administration in order to investigate the causal role of cortisol.

Finally, I investigated these processes not only in healthy participants characterized by high versus low levels of trait avoidance/inhibition, but also in two samples of patients with a clinical diagnosis of SAD. Student samples with high self-reported, but non-clinical, levels of social or trait anxiety are widely used in anxiety research, and can provide a valuable contribution to the understanding of basic processes implicated in anxiety disorders. Nevertheless, studies in clinical populations are necessary to draw conclusions about the generalizability of these findings as well as the clinical significance of these processes.

Overview of chapters

Chapter 2 describes a first study testing the predictions that a) individuals characterized by high levels of behavioral inhibition show preferential processing of and stronger avoidance tendencies towards social threat cues, and b) that these processes are facilitated by cortisol. This is investigated by measuring the effects of cortisol administration on the AA- task in a sample of pre-selected high and low behaviorally inhibited/anxious students, using a placebo-controlled within-subject design. Furthermore, event-related potentials (ERPs) are measured during task performance to gain more insight in the brain processes associated with threat processing and approach and avoidance reactions.

Chapter 3 investigates the effects endogenous cortisol increases on approach-avoidance behavior. Therefore, the Trier Social Stress Test (Kirschbaum, Pilke, &

Hellhammer, 1993) is administered to SAD patients, and performance on the AA-task in this psychosocial stress condition is compared to baseline using a within-subject design. The possible role of hypercortisolism in the failing regulation of social fear and fear behavior in SAD is investigated by directly relating the stress-induced cortisol responses to AA-task performance. A sample of matched healthy participants and a sample of patients with Post-Traumatic Stress Disorder (PTSD) are included as control groups to investigate the specificity of the effects.

Following the study in *Chapter 3*, the study in *Chapter 4* aims to get a better understanding of the *causal* role of cortisol, as well as of the neural processes involved in the regulation of social fear behavior in SAD. Furthermore, the same experimental procedure is used as in *Chapter 2*, to test whether the findings in high inhibited/anxious healthy participants generalize to a clinical population. Therefore, in this study the effects of cortisol administration on performance of the AA-task are measured in a second sample of patients with SAD, using a placebo controlled within-subject design, and with ERPs measured during task performance.

In *Chapter 5* the hypothesis is tested that patients with SAD show increased early processing of angry faces regardless of whether this is required for task performance, and even under conditions of restricted stimulus awareness. Furthermore, as effects of cortisol have been shown to be context-dependent, I investigate whether the effects of cortisol on threat processing are similar when the stimulus emotion is implicit (task-irrelevant), compared to when stimulus emotion is explicit and task relevant, as in the AA-task. In this study, the effect of cortisol administration on reaction times and ERPs is measured in patients with SAD during color-naming of masked and unmasked emotional faces in a modified emotional Stroop task, using a placebo-controlled within-subject design. This study is conducted in the same participant sample as *Chapter 4*.

In *Chapter 6*, a more theoretical-methodological issue is explored, namely to which extent the approach-avoidance effects, as measured in previous chapters, depend on the *actions* of the participants themselves or may be mediated by a representation of relative distance between the participant and the stimulus (e.g., Neumann & Strack, 2000). In a series of four reaction time experiments, the effects of stimulus movements on the evaluation of happy and angry face stimuli are investigated in healthy male and female students. It is predicted that changes in relative distance due to stimulus

movement exert similar effects on affective evaluation as the approach and avoidance movements executed by the participant in the AA- task.

Chapter 7 describes the effects of cortisol administration and individual differences in trait avoidance/behavioral inhibition on resting state brain activity that has been associated with approach and avoidant motivational states.

Finally, *Chapter 8* presents an overview and integration of the findings of the *Chapters 2* to *7*, and a discussion of the strengths and limitations of these studies. The chapter concludes with suggestions for future research and implications for clinical practice.

Chapter 2

The effects of cortisol administration on approach-avoidance behavior: An event-related potential study.

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J. M. van Peer, K. Roelofs, M. Rotteveel, J.G. van Dijk, Ph. Spinhoven, & K. R. Ridderinkhof

Abstract

We investigated the effects of cortisol administration (50 mg) on approach and avoidance tendencies in low and high trait avoidant healthy young men. Event-related brain potentials (ERPs) were measured during a reaction time task, in which participants evaluated the emotional expression of photographs of happy and angry faces by making an approaching (flexion) or avoiding (extension) arm movement. The task consisted of an affect-congruent (approach happy faces and avoid angry faces) and an affect-incongruent (reversed instruction) condition. Behavioral and ERP analyses showed that cortisol enhanced congruency effects for angry faces in highly avoidant individuals only: The ERP effects involved an increase of both early (P150) and late (P3) positive amplitudes, indicative of increased processing of the angry faces in high avoidant subjects after cortisol administration. Together, these results suggest a context specific effect of cortisol on processing of, and adaptive responses to, motivationally significant threat stimuli, particularly in participants highly sensitive to threat signals.

Introduction

Activity of the Hypothalamus-Pituitary-Adrenal (HPA) axis is important in the regulation of adaptive stress responses such as the generation of active avoidance reactions (see Sapolsky et al., 2000). Stress leads to activation of the HPA system, resulting in the release of endogenous glucocorticoids such as cortisol. Particularly when measured in social situations, elevated cortisol levels have been found to be related to the manifestation of social submissiveness and avoidance behavior (Sapolsky, 1990). Despite the extensive literature on the relation between HPA-axis activity and avoidance behavior in animals, little is known about the role of cortisol in the generation of human avoidance behavior. In this study, we examined the effect of cortisol administration on avoidance reactions to threatening social stimuli (angry faces) in human participants. In addition, to gain more insight in the brain processes underlying these reactions, we measured event-related brain potentials (ERPs) during performance of an approach-avoidance task, specifically focusing on positive components related to emotional face processing.

The generation of active avoidance responses depends on a motivational network that involves various brain regions (see LeDoux, 2002; Rolls, 2000). When threat stimuli are processed by the amygdala, direct autonomic responses and primary motor reactions such as freezing are activated via connections to the brainstem. Moreover, motivational systems are activated that guide instrumental responses based on past learning or instantaneous decisions. The hippocampus and prefrontal cortex (PFC) play an important role in these motivational systems. The PFC is thought to integrate information on arousal (from brainstem centers) with context-relevant information (from the hippocampus) and with temporary contents of working memory (from PFC areas) in controlling motor responses (via connections with the motor cortex). The anterior cingulate (ACC) and orbitofrontal (OFC) regions of the PFC in particular are involved in these motivational systems, which enable approach and avoidance reactions to emotional stimuli (see LeDoux, 2002; Roelofs et al., 2009b; Rolls, 2000).

Rolls (2000) stressed the importance of processing of facial expressions by these motivational systems. Emotion has a communicative function, and faces constitute important signals of threat or appeasement in the social environment. In a series of lesion studies, Hornak et al. (2003) showed that in human participants both the OFC and

the ACC are involved in emotion processing, including the identification of facial expression, social behavior, and subjective emotional state.

Angry facial expressions are commonly used as social threat stimuli in human research on threat processing. Neuroimaging studies have shown that viewing angry faces activates large parts of the above mentioned motivational network, with the ACC, OFC, and amygdala in particular (for an overview see Adolphs, 2002; McClure et al., 2004; Strauss et al., 2005). In addition, transcranial magnetic stimulation of the medial PFC/ACC has been found to disrupt the processing of angry facial expressions (Harmer, Thilo, Rothwell, & Goodwin, 2001). Adolphs (2002) argued that whereas activation of the amygdala appears to depend on relatively passive or implicit processing of the emotion (such as in passive viewing paradigms), prefrontal regions may be activated more when participants are engaged in a cognitive task requiring explicit identification of the emotion, which in turn may inhibit the amygdala's activation.

ERP studies have also indicated that prefrontal motivational networks are involved in the processing of facial expressions. An enhanced positivity in response to emotional relative to neutral faces has been found over prefrontal areas as early as 120 ms after stimulus presentation (Eimer & Holmes, 2002) or between 160 and 215 ms (Eimer, Holmes, & McGlone, 2003). This suggests that cortical circuits involved in the detection of emotionally significant events can be triggered rapidly by emotional facial expressions (Eimer et al., 2003; Pizzagalli, Regard, & Lehmann, 1999; Sato, Kochiyama, Yoshikawa, & Matsumura, 2001). In addition, a more broadly distributed positivity (over parietal as well as frontal and central areas) has been observed beyond 300 ms (Eimer et al., 2003). In particular faces signaling threat (i.e., fearful or angry faces as opposed to happy or neutral faces) have been found to show these enhanced amplitudes in both early (e.g., 50-250 ms: Ashley, Vuilleumier, & Swick, 2004; Bar-Haim, Lamy, & Glickman, 2005; Schupp et al., 2004; Williams, Palmer, Liddell, Song, & Gordon, 2006) and late positive components (300-500 ms: Schupp et al., 2004; Williams et al., 2006). Interestingly, recent studies reported the ERP effects of emotional expressions to be attention dependent (Eimer et al., 2003; Krolak-Salmon, Fischer, Vighetto, & Mauguière, 2001), suggesting they may reflect a greater allocation of attention to motivationally relevant input (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000).

In sum, a frontolimbic motivational network is involved in the processing of social threat stimuli and the generation of avoidance behavior. In the next section we

explore how the stress hormone cortisol, which is thought to be important in the generation of adaptive stress responses (e.g., Sapolsky et al., 2000), may affect this network and, consequently, approach and avoidance behavior. It is well established that not only the hippocampus but also the PFC is a target structure for cortisol (e.g., Meaney & Aitken, 1985; Radley et al., 2004). Exogenously administered cortisol has been shown to affect prefrontal functions, such as working memory, in humans (for a review see Wolf, 2003). In addition, there is increasing evidence from animal studies that PFC mediated avoidance behavior and fearful temperament are positively correlated with high levels of cortisol (see e.g., Kalin et al., 1998a, 2000; Kalin, Shelton, Rickman, & Davidson, 1998). De Kloet et al. (1999) emphasized that glucocorticoids influence information-processing systems conditionally, so that specific internal and external stimuli are more likely to elicit responses in the appropriate context. In this way, information processing is biased towards adaptive behavior that is most relevant to the situation.

Human studies on the relation between cortisol, the processing of social threat stimuli and avoidance behavior are scarce, but a recent study by Putman, Hermans and Van Honk (2007) suggested that acute (25 mg) cortisol administration enhanced preferential processing of angry faces in healthy young men. The results of this study showed a significant increase in memory bias for angry faces (i.e., enhanced spatial working memory performance compared to neutral faces) after cortisol administration compared to placebo. No such memory bias was found for happy faces. In addition, a study by Van Honk et al. (1998) in which angry and neutral faces were presented in a Stroop paradigm indicated that increased basal cortisol levels were associated with faster responses to angry faces, which was interpreted as reflecting (adaptive) avoidance. However, no studies so far have addressed the effects of cortisol administration on overt avoidance behavior.

A systematic and objective method to study human avoidance behavior was provided by Solarz (1960) and Chen and Bargh (1999), consisting of a reaction time task in which individuals evaluate the emotional valence of positive and negative word stimuli by making arm movements (arm flexion or extension) that are either congruent or incongruent with their intuitive action tendencies. Rotteveel and Phaf (2004) extended this paradigm to the nonverbal domain, using pictures of happy and angry faces (the approach-avoidance (AA) task). Affect-congruent movements involve arm

flexion (approach) in response to a positive stimulus (happy face) and arm extension (avoidance) in response to a negative stimulus (angry face). Affect-incongruent movements involve reversed mapping instructions (from stimulus valence to arm movement) that conflict with participants' intuitive action tendencies (i.e., to approach positive and avoid negative stimuli). With this paradigm a congruency effect is typically found, indicating faster responses for affect-congruent arm movements compared to affect- incongruent arm movements (see also Chen & Bargh, 1999; Markman & Brendl, 2005; Solarz, 1960).

Using this AA task, Roelofs et al. (2005) found an effect of stress-induced cortisol responses on the congruency effects. Participants with relatively high stress-induced cortisol responses (high CR) showed increased AA congruency effects when tested in baseline conditions, but no significant congruency effects during stress. In contrast, for low CR participants the congruency effects were only significant during and not before stress. Thus, the results of this study showed a significant interaction of cortisol response and stress on approach-avoidance tendencies as measured by the AA task. However, the effects of high stress-induced cortisol levels could not be disentangled from the influence of individual differences in stress-responsiveness or the effect of the social stress context. Therefore, the present study aimed to further investigate the effects of high cortisol levels on approach-avoidance tendencies, by studying the effects of cortisol *administration* on behavioral responses (particularly threat avoidance) in the AA task.

In addition, to investigate the effects of individual differences in threat sensitivity on behavioral responses to the threat signaling angry faces in the AA task, we compared participants with high scores to participants with low scores on a self-report measure of threat sensitivity (the Behavioral Inhibition Scale [BIS]: Carver & White, 1994). Individuals with high scores on this scale (high BIS participants) can be characterized as anxiety prone, and tend to avoid threat (Carver & White, 1994). Compared to low BIS participants, we expected high BIS participants to be particularly responsive to social threat cues and to show relatively increased avoidance tendencies to the angry faces.

To test the effects of cortisol on these avoidance reactions, we administered the AA task to both participant groups after placebo and cortisol (hydrocortisone) administration. Because high cortisol levels have been associated with context-relevant adaptive responses (De Kloet et al., 1999; Sapolsky et al., 2000), biased processing of

angry faces (Putman et al., 2007a), and increased avoidance responses to threat (Buss et al., 2003; Kalin et al., 1998a, 1998b, 2000; Van Honk et al., 1998), we expected cortisol administration to result in relatively increased avoidance reactions to angry faces on the AA task. Furthermore, we hypothesized that this effect would be especially strong for the high BIS subjects, given their increased sensitivity to these social threat cues. Such increased threat avoidance in the AA task can be either manifested by an increase in the effect of arm movement (faster avoidance than approach movements) for angry faces, or an increase in the effect of emotional expression for avoidance reactions (faster avoidance of angry than happy faces).

The second purpose of this study was to investigate brain processes associated with these effects using ERPs, with specific focus on components involved in emotional face processing and action monitoring. ERP components of particular interest were the previously mentioned positive waves that have been found over the prefrontal cortex between 120 and 250 ms post-stimulus, and the more broadly distributed positive wave observed beyond 300 ms (e.g., Eimer et al., 2003; Schupp et al., 2004). In line with our behavioral expectations, we expected cortisol administration to result in increased amplitudes of these components especially in the high avoidant (high BIS) participants during avoidant reactions to angry faces.[1]

A final component of interest was the N2, a frontocentral negative wave arising 200-350 ms post-stimulus. The N2 has been found to be increased in high conflict conditions, when incompatible response tendencies are simultaneously activated, and is suggested to reflect action monitoring (e.g., Van Veen & Carter, 2002), a function served by the medial prefrontal cortex (Ridderinkhof, Ullsperger, Crone, & Nieuwenhuis, 2004). In the AA task such response conflict may be elicited by affect-incongruent trials where the executed response is hypothesized to be in conflict with the participants' intuitive response tendency (i.e., to approach happy and avoid angry faces) (see Chen & Bargh, 1999; Rotteveel & Phaf, 2004). This study allows exploring whether the AA task indeed elicits significant N2 effects and whether cortisol administration may affect action monitoring during the generation of approach-avoidance responses.

To summarize our major predictions, we expected that cortisol administration

[1] In contrast, the face-specific N170 component, which can be recorded over posterior temporal areas, has been found to be relatively insensitive to emotion processing and is predominantly associated with structural encoding of faces (see e.g., Ashley et al., 2004). We therefore had no predictions regarding this component with relevance to approach and avoidance behavior.

would result in a facilitation of threat avoidance in high BIS participants. In addition, these behavioral effects were expected to be accompanied by increased amplitudes of ERP components involved in emotional face processing (in particular social threat). Finally, we explored whether cortisol administration would also increase action monitoring in high BIS participants.

Methods

Participants

Forty male students recruited from the University of Leiden participated in the experiment for financial (i.e., 40 euros) or course credit. To create two extreme groups that differed in threat sensitivity, we selected a priori 20 students with low scores (≤ 16) and 20 students with high scores (≥ 21) on the Behavioral Inhibition Scale (BIS: Carver & White, 1994, see trait measures). Cutoff scores for these groups were based on the lower third and the upper third of the distribution of BIS scores (range 9-28, M = 18.5, SD = 3.6) in a sample of 153 male students.

Participants in this study were screened with the General Health Questionnaire (GHQ-12: M = 1.45, SD = 1.69); Goldberg, 1978; Dutch version: Koeter & Ormel, 1991) and a biographic questionnaire to exclude any psychiatric disorder, clinical significant medical disease, past head injury with loss of consciousness > 5 min, and use of medication. Inclusion criteria were right-handedness, normal or corrected-to-normal vision, age 18-30, and bodyweight 60-85 kg. Participants were instructed to minimize physical exercise, not to take large meals, chocolate or caffeine during the morning preceding the experiment, and not to eat, drink low pH drinks or smoke cigarettes in the hour before the start of the experiment, because these variables can affect saliva cortisol measurements. All participants provided written informed consent prior to participation in the study, which was approved by the Medical Ethical Committee of the Leiden University Medical Center.

Materials and procedure

All participants were tested in a hydrocortisone (50 mg) and a placebo condition in a double-blind, within-subject crossover design. The order of cortisol or placebo administration (i.e., a capsule) was random and balanced within the high and low BIS

Figure 2.1. Examples of a happy and angry face stimulus used in the AA task.

groups. The two experimental sessions were one week apart. On the days of testing, participants arrived at the laboratory at either 12.15 or 2.15 p.m. After a short introduction, drug administration followed at 12.30 or 2.30 p.m., respectively. After ingestion of the capsule, a resting period of 1 h followed to allow for the cortisol to take effect. During this period, participants completed questionnaires and practiced with the response device for the approach-avoidance task, after which the electrodes for the electrophysiological measurements were placed. Subsequently, the experiment started with a short recording (~15 min) of the electroencephalogram (EEG) during rest, after which the approach-avoidance task was administered, followed by a number of additional cognitive tests of which the results will be reported elsewhere. During task performance, participants sat in an air-conditioned and sound-attenuated room in front of a computer monitor, and the experimenter sat in an adjacent room, where the EEG apparatus was located.

Approach Avoidance task

In this affect-evaluation task (Rotteveel & Phaf, 2004), 60 pictures with facial expressions from Ekman and Friesen (1976), Matsumoto and Ekman (1988), and Lundqvist, Flykt, and Öhman (1998) served as stimuli. Half of the pictures were taken from female and the other half from male models (total of 30 models). Pictures consisted of grayscale photographs presented against a black background (see Figure 2.1). To minimize variation in physical parameters unrelated to emotional expression, both the

happy and the angry expression were taken from the same model. In addition, each face was trimmed to exclude the hair and non-facial contours, and adjusted to match for size, brightness and contrast. Each picture measured 12.4 cm × 8.9 cm (*h* × *w*), and was presented at the center of a 15 in. computer screen at 70 cm viewing distance, resulting in a 10.1° × 7.3° visual angle.

The start of an individual trial was indicated by the appearance of a central fixation point (lasting 100 ms). After an interval of 300 ms the stimulus was presented for 100 ms. The time interval between successive stimuli was randomized between 1500 and 2500 ms. Pictures were presented using the Wesp Experimentation Stimulus Program (version 1.98 WESP XP, Molenkamp, University of Amsterdam, 2002).

Responses were registered by means of three buttons (of 16 cm^2) that were fixed to a vertical stand (see Rotteveel & Phaf, 2004, Figure 1). Participants sat to the left of the stand, allowing them to respond with their right hand. For the resting position participants were instructed to push the home button (fixed in the middle) loosely with the back of their right hand as long as no response was given. The height of this button was set for each participant individually, such that the angle between their forearm and upper arm was 110° in the resting position. In this position both the biceps and the triceps were equally tensed. The response buttons were positioned above and below the home button (at a distance of 10.3 cm). This allowed participants to simply flex or extend their right arm in responding without the need for precise aiming at the response buttons.

Participants were verbally instructed to evaluate the facial expressions (i.e., happy or angry), and to respond as fast and accurate as possible to the stimuli by releasing the home button and pressing one of the response buttons. After this, they had to return their hand to the home button. Participants received alternately an affect-congruent or an affect-incongruent instruction. The affect-congruent instruction indicated pressing the upper response button (i.e., arm flexion) for happy faces and the lower button (i.e., arm extension) for angry faces. In the affect-incongruent condition the mapping of the facial expression to the response buttons was reversed. No reference was made in the instructions to congruence and incongruence, approach and avoidance, or arm flexion and extension.

The task consisted of four series of 60 trials. Within each series all stimuli were presented once in a semi randomized order (with a maximum of 3 happy or angry and 3

male or female pictures in succession). Half of the participants started with a series with an affect-congruent instruction, followed by a series with an affect-incongruent instruction, another affect-congruent instruction series, and a final affect-incongruent instruction series. The other half of the participants received the reversed order of instructions. Between each series participants performed an unrelated working memory task (digit span or spatial memory) that served to ease the transition from affect-congruent to affect-incongruent instruction or vice versa. Each of the four series was divided into three blocks of 20 trials, with a short break (~ 30 s) between blocks, and was preceded by 20 practice trials of stimuli that were not included in the experimental series.

The task provided three behavioral measures: error rates (percentage incorrect responses) and two reaction time (RT) measures. The initiation time (IT) is the time between stimulus onset and the release of the home-button. The movement time (MT) is the time between the release of the home button and the pushing of the response button. IT constitutes an index of central processes reflecting stimulus evaluation, response selection and programming the execution of movements, and is relatively independent of MT, which reflects the magnitude of the neuro-muscular response (Fitts, 1954). The influence of affect on the reaction times is therefore primarily expected in IT, rather than MT (see Rotteveel & Phaf, 2004; Solarz, 1960). Incorrect responses and RTs that deviated more than 2.5 *SD* from the individual RT averages per cell (cells defined by cortisol condition × emotion × arm movement) were excluded from the RT analyses.

Electrophysiological recording and analysis

The electroencephalogram (EEG) was recorded from 19 scalp locations according to the international 10-20 system and referred on-line to C3/C4. An average earlobe reference was derived off-line. Vertical electro-oculogram (EOG) was recorded bipolarly from the supraorbital and the infraorbital ridge of the right eye, and horizontal EOG from the outer canthi of both eyes. The ground electrode was located at Fpz. EEG impedances were kept below 5 kΩ. The EEG and EOG signals were digitized at 500 Hz. Signals were processed off-line using Brain Vision Analyzer software (version 1.05, Brain Products GmbH, 1998-2004). Codes synchronized to stimulus presentation and response were used to allow offline averaging of epochs associated with specific stimulus and response types. The epoch ran for 1000 ms, beginning 200 ms prior to

stimulus onset, aligned to a 100 ms prestimulus baseline. Single trials were corrected for the effects of eye blinks and eye movements using a standard procedure (Gratton, Coles, & Donchin, 1983). Data were subsequently filtered digitally with a 0.1 Hz high-pass filter, a 35 Hz low-pass filter (both with a roll-off of 12 dB/oct) and a 50 Hz notch filter. After baseline correction, trials including amplitude values larger than ±75μV, a difference >100 μV between the lowest and the highest amplitude within the segment, a period >100 ms with activity <0.50 μV, or a difference >50 μV between two subsequent sampling points were considered artifacts and were excluded from analyses (9% of total data set). We analyzed stimulus-locked data only for trials with correct responses with reaction times between 150 and 1000 ms, computing averages for each category (defined by emotion × arm movement). After rejection of artifacts and incorrect responses a mean number of 49.7 trials (*SD* = 9.4) per category was left for each participant in each cortisol condition for further analysis. To facilitate peak detection, individual averages per category were low pass filtered at 12 Hz before peaks were identified and measured. The following stimulus-locked ERP components (peak amplitudes relative to baseline) were identified at electrodes F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4: N1 (the first major negative wave occurring 30-130 ms post-stimulus), followed by P150 (the first major positive wave occurring 120-200 ms post-stimulus), N2 (180-300 ms), and P3 (270-400 ms). Time windows for peak detection were based on visual inspection of the grand average ERPs, averaged across all participants and categories.

Trait measures

As described above, participants were assigned to two groups based on their score on the Behavioral Inhibition Scale (BIS).[2] This 7-item self-report scale measures sensitivity to signals of threat and was shown to have good reliability (BIS/BAS: Carver & White, 1994). Items are statements that reflect a concern over the possibility of a bad occurrence or a sensitivity to such events when they do occur, and each item is rated on a four-point scale, with a maximum total score of 28. The Behavioral Activation Scale (BAS) consists of a total of 13 items measuring sensitivity to reward. In addition, we

[2] The BIS/BAS scales of Carver and White (1994) were developed on the basis of the Reinforcement Sensitivity Theory (RST: e.g., Gray, 1982). Note that due to a recent revision of this theory (Gray & McNaughton, 2000) the BIS scale, designed to measure threat sensitivity, is likely associated with the Fight Flight and Freezing System in the revised RST (Smillie et al., 2006).

administered questionnaires measuring trait anxiety (State Trait Anxiety Inventory [STAI]: Spielberger, 1983; Dutch version: Van der Ploeg, 2000) and social anxiety (Social Phobia and Anxiety Inventory [SPAI]: Turner, Beidel, Dancu, & Stanley, 1989; Dutch version: Bögels & Reith, 1999), as well as the temperament subscales of the Temperament and Character Inventory (TCI), which contains a Novelty Seeking and Harm Avoidance subscale that have been related to behavioral activation and behavioral inhibition, respectively (Cloninger, Przybeck, Svrakic, & Wetzel, 1994; Dutch version: De la Rie, Duijsens, & Cloninger, 1998).

Cortisol and subjective measures

Saliva samples were obtained using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Samples were obtained at four assessment points over a 165 min period, at respectively -5 min (T0), +60 min (T1), +120 min (T2), and +160 min (T3) with reference to capsule ingestion. Biochemical analysis of free cortisol in saliva was performed using a competitive electrochemiluminescence immunoassay (ECLIA, Elecsys 2010, Roche Diagnostics), as described elsewhere (Van Aken, Romijn, Miltenburg, & Lentjes, 2003).

Self-reported mood (tension, fatigue, depression, anxiety, and activation at T0, T1, and T3) and motivation and concentration (directly before and after the AA-task) were rated on 10 cm visual analogue scales (VAS). In addition, state anxiety (STAI-state: Spielberger, 1983) was measured at T0 and T3.

Statistical analyses

The influence of cortisol administration on subjective measures, salivary cortisol, AA-task performance, and ERP peak amplitudes were tested with repeated measures analyses of variance (ANOVAs rm) using the Statistical Package for the Social Sciences (SPSS 14.0, SPSS Inc., 1989-2005). All statistical analyses described employed a two-tailed alpha of .05. Effect sizes are reported as proportion of explained variance (partial eta squared [η^2]). Reaction times of two participants (both from the low BIS group) were not registered due to technical problems. These participants were excluded from all analyses, resulting in a total number of 18 subjects in the low BIS group.

Table 2.1. Trait scores for low BIS and high BIS groups

	Low BIS		High BIS	
Measure	*M*	*SD*	*M*	*SD*
Age	20.4	1.7	19.9	1.4
BMI	21.4	1.6	21.6	1.7
BIS***	14.0	2.1	22.1	1.7
BAS total	37.9	4.4	37.9	3.4
STAI-trait***	29.6	4.9	37.6	4.1
SPAI Total **	40.4	17.5	57.8	13.0
TCI				
Harm avoidance***	4.1	3.4	9.4	3.0
Novelty seeking	9.5	4.2	8.9	3.4
Reward dependence	8.7	2.5	10.0	2.7
Persistence	1.8	1.6	2.2	1.2

Note: BMI = Body Mass Index; BIS = Behavioral Inhibition Scale; BAS = Behavioral Activation Scale; STAI = State Trait Anxiety Inventory; SPAI = Social Phobia and Anxiety Inventory; TCI = Temperament and Character Inventory. $^{**}p < .01$ $^{***}p < .001$.

Results

Trait measures

Table 2.1 presents the mean values for the low and the high BIS groups on the trait measures. As expected, and due to our selection procedure, groups differed significantly on BIS-scores ($F(1,36) = 177.87$, $p < .001$, $\eta^2 = 0.83$). In addition, the high BIS group scored significantly higher on several anxiety measures: trait anxiety (STAI-T: $F(1,36) = 30.18$, $p < .001$, $\eta^2 = 0.46$), social anxiety (SPAI total: $F(1,35) = 11.31$, $p < .01$, $\eta^2 = 0.26$) and harm avoidance (TCI-HA: $F(1,36) = 26.01$, $p < .001$, $\eta^2 = 0.42$). The groups did not differ significantly in age, body mass index or any of the other trait measures (all $p > .10$).

Cortisol and subjective measures

Salivary cortisol

Salivary cortisol (nmol/L) measures (see Table 2.2) were skewed and therefore log transformed before statistical analysis. The results of a 2 (group: low BIS, high BIS) × 2 (cortisol: placebo, cortisol) × 4 (time: T0, T1, T2, T3) ANOVA rm yielded a significant interaction of cortisol × time ($F(3, 102) = 188.92$, $p < .0001$, $\eta^2 = 0.98$). This result indicates that, as expected, unbound levels of cortisol did not differ between conditions before capsule intake (T0: $F(1,35) = 0.44$, $p = .51$), but were significantly increased after cortisol administration compared to placebo from 1 h after capsule intake (T1: $F(1,35) =$

Table 2.2. Mean free salivary cortisol levels (in nmol/L) after placebo and cortisol administration relative to time of capsule intake (t = 0)

	Placebo		Cortisol	
Time (min)	*M*	*SD*	*M*	*SD*
- 5	8.9	3.2	9.1	2.8
+ 60***	6.8	1.7	161.7	145.1
+ 120***	6.7	3.5	122.5	55.0
+ 165***	6.5	2.6	112.4	50.2

Note: N = 36 due to missing values of two participants.***p < .001

320.48, p < .0001, η^2 = 0.90) until the end of the experiment (T2: F(1,34) = 846.67, p < .0001, η^2 = 0.96; T3: F(1,35) = 1265.77, p < .0001, η^2 = 0.97). There were no significant differences in salivary cortisol values between groups.

Subjective measures

To investigate group differences in subjective mood during task administration and effects of cortisol administration on mood, we conducted separate ANOVAs rm with group (low BIS, high BIS) × cortisol (placebo, cortisol) × time for STAI-state (T0, T3) and VAS (T0, T1, T3) tension, fatigue, depression, anxiety, and activation. Results showed significant main effects of group on STAI-state anxiety (F(1,36) = 8.49, p < .01, η^2 = 0.19) and VAS tension (F(1,36) = 7.23, p < .05, η^2 = 0.17) indicating higher scores for the high BIS group (STAI-S: M = 33.5; TEN: M = 2.7) compared to the low BIS group (STAI-S: M = 28.8; TEN: M = 1.9). In addition, VAS anxiety scores tended to be higher for high BIS (M = 1.6) compared to low BIS (M = 1.3) participants (F(1,36) = 4.06, p = .051, η^2 = 0.10. There were no significant main or interaction effects of cortisol on mood.

Behavioral results

To investigate the influence of cortisol administration and trait avoidance on performance of the AA-task we conducted separate 2 (group: low BIS, high BIS) × 2 (cortisol: placebo, cortisol) × 2 (emotion: happy, angry) × 2 (arm movement: approach (flex), avoid (extend) ANOVAs rm on error rates and reaction times (MT and IT).[3] For all behavioral measures we will first present results concerning the AA congruency-effect (i.e., the emotion × arm movement interaction) and subsequently the effects of group and cortisol on this congruency effect.

[3] We performed two additional analyses, with session (first day, second day) and stimulus gender (male, female) as additional factors. Since both analyses revealed no significant effects of these factors on the emotion × arm movement interaction, we have further left them out of the analyses.

Error rates

As to be expected in the AA-task (see Rotteveel and Phaf, 2004), a significant emotion × arm movement interaction ($F(1,36) = 5.77$, $p < .05$, $\eta^2 = 0.14$) showed that participants made more errors (%) during affect-incongruent arm movements (avoid happy: $M = 7.2$; approach angry: $M = 8.8$) than affect-congruent arm movements (approach happy: $M = 5.3$; avoid angry: $M = 7.8$). There were no effects of group or cortisol on these congruency effects (all $p > .16$).

Initiation times (IT)

Also for the IT (in ms), we found the expected AA congruency effect. A significant emotion × arm movement interaction ($F(1,36) = 21.05$, $p < .0001$, $\eta^2 = 0.37$) showed that participants were faster in initiating affect-congruent (approach happy: $M = 486$; avoid angry: $M = 500$) than affect-incongruent arm movements (avoid happy: $M = 506$; approach angry: $M = 511$). The effects of group or cortisol on this emotion × arm movement interaction were not significant.

We did, however, find a significant three-way interaction of group × cortisol × arm movement ($F(1,36) = 15.03$, $p < .0001$, $\eta^2 = 0.29$). Separate ANOVAs for the high and low BIS group showed a significant cortisol × arm movement interaction for the high BIS group ($F(1,19) = 16.11$, $p < .001$, $\eta^2 = 0.46$), but not for the low BIS group ($F(1,17) = 2.25$, $p = .15$) (see Figure 2.2). The significant results for the high BIS group were due to a significant effect of cortisol on the approach movement ($F(1,19) = 5.76$, $p < .05$, $\eta^2 = 0.23$), indicating that approach reactions were slowed after cortisol administration in high BIS participants. The cortisol × *emotion* × arm movement interaction was not significant in the high BIS group ($F(1,19) = 0.38$, $p = .55$), indicating that the cortisol × arm movement interaction was not different for happy and angry faces. However, because we had specific hypotheses about this effect for angry faces, we additionally checked whether the cortisol × arm movement interaction for the high BIS group would hold when tested for responses to angry faces only. The results indeed showed the cortisol × arm movement interaction in the high BIS group to be significant for angry faces ($F(1,19) = 10.30$, $p < .01$, $\eta^2 = 0.35$). Interestingly, this effect was not significant for happy faces (cortisol × arm movement: $F(1,19) = 2.16$, $p = .16$). In addition, due to the slowing of approach reactions after cortisol administration, the congruency effect for angry faces (i.e., faster avoidance than approach) was only significant for the high BIS

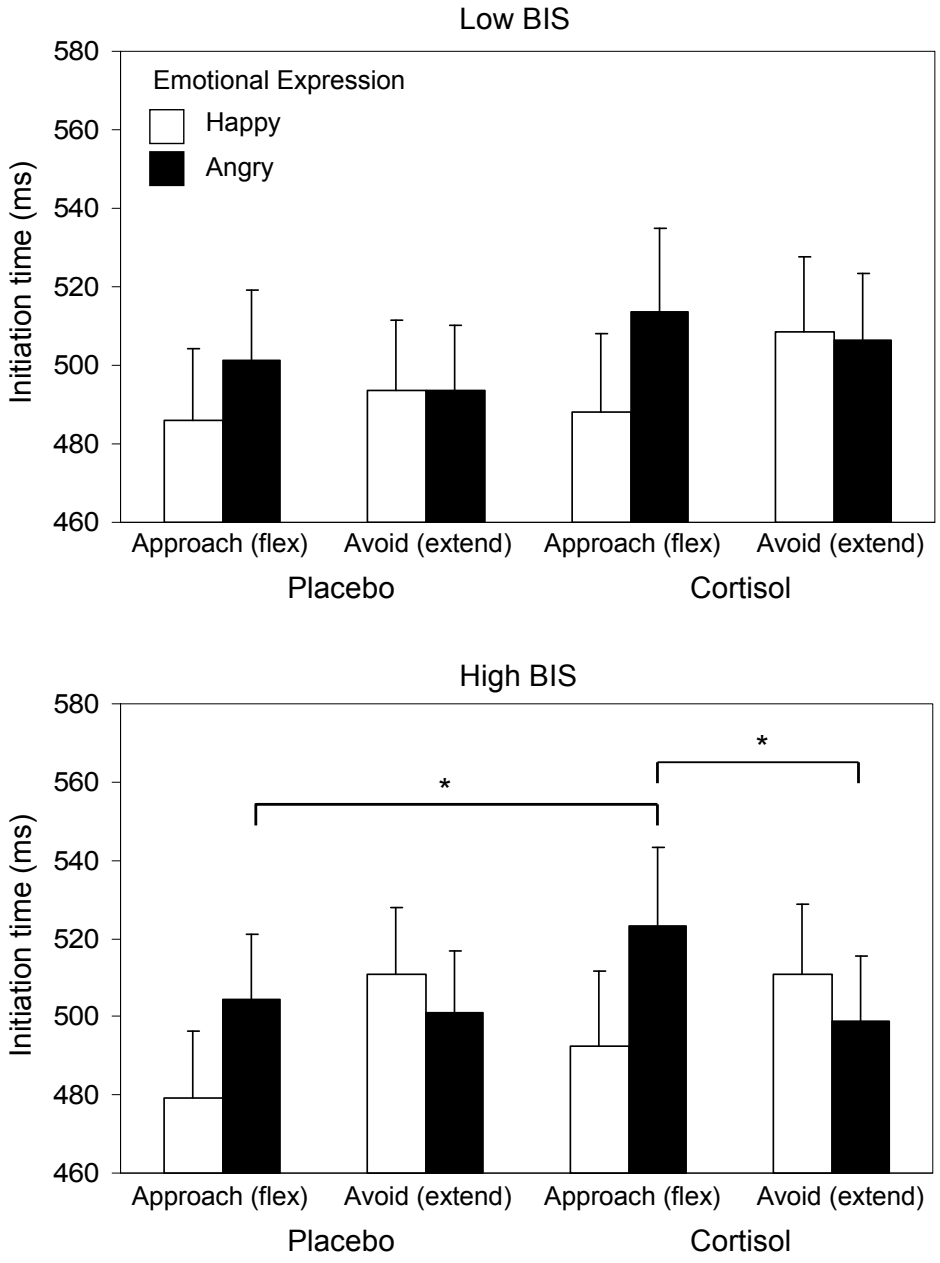

Figure 2.2. Mean initiation times (in ms) on the AA task for the low BIS (upper panel) and high BIS (lower panel) group after placebo and cortisol administration. Cortisol administration resulted in a significant slowing of approach, but not avoidance, movements in the high BIS group only. This effect was significant for angry faces, but not for happy faces. The congruency effect for angry faces (i.e. faster avoidance than approach) was only significant in the high BIS group after cortisol administration. Error bars indicate the standard errors of the means. *p < .05.

group in the cortisol condition (arm movement: $F(1,19) = 8.84$, $p < 0.01$, $\eta^2 = 0.32$) and not the placebo condition ($F(1,19) = 0.17$, $p = .69$) (see Figure 2.2).

Thus, in line with our hypotheses, cortisol administration affected approach-avoidance congruency effects especially to angry faces in high BIS participants. Although the results indicated that this effect did not differ significantly between happy and angry faces, the effect was only significant for angry, and not for happy faces.

Movement times (MT)

Like the error rates and IT, the MT (in ms) showed a significant emotion × arm movement interaction ($F(1,36) = 8.48$, $p < .01$, $\eta^2 = 0.19$) indicating faster execution of affect-congruent (approach happy: $M = 138$; avoid angry: $M = 134$) than affect-incongruent arm movements (avoid happy: $M = 140$; approach angry: $M = 142$). There were no effects of group or cortisol on these congruency effects in MT.

Event-related potentials

The data from the Cz electrode appeared most representative for the 3 midline electrodes (Fz, Cz, Pz) and are presented in Figure 2.3. The general morphology of the waveform at these midline electrodes included a prominent, early negative peak at 100 ms (N1), followed by a positive wave at 150 ms (P150), a second negative wave at 230 ms (N2) and a final positive wave at 350 ms (P3). As shown in Figure 2.3, event-related peaks were pronounced.

Baseline-to-peak amplitudes were analyzed with separate 2 (group: low BIS, high BIS) × 2 (cortisol: placebo, cortisol) × 2 (emotion: happy, angry) × 2 (am movement: approach (flex), avoid (extend) × 3 (electrode: Fz, Cz, Pz)[4] ANOVAs rm for N1, P150, N2, and P3. As for the behavioral results, we will focus on the AA congruency-effects (i.e., the emotion × arm movement interaction) and the effects of group and cortisol on this interaction.

P150

For P150 peak amplitude, we found a significant four-way interaction of group × cortisol × emotion × arm movement ($F(1,36) = 4.94$, $p < .05$, $\eta^2 = 0.12$). Follow up analyses to determine the nature of this interaction showed that the emotion × arm condition ($F(1,19) = 6.50$, $p < .05$, $\eta^2 = 0.26$) (see Figure 2.4, panel A). It was not significant for the high BIS group in the placebo condition ($F(1,19) = 0.17$, $p = .69$), nor was it significant for the low BIS group in either the cortisol condition ($F(1,17) = 1.88$, $p = .19$) or the placebo condition ($F(1,17) = 0.42$, $p = .53$). Further analyses of this emotion × arm movement interaction for the high BIS group in the cortisol condition revealed that the effect of arm movement was significant for angry faces ($F(1,19) = 9.93$, $p < .01$,

[4] A second analysis for each component was conducted with F3, F4, C3, C4, P3, and P4 as additional electrodes, and with laterality (left, midline, right) as an additional factor. This analysis confirmed the conclusion based on visual inspection that there were no laterality effects involving emotion × arm movement. Therefore, only results of midline electrodes are presented.

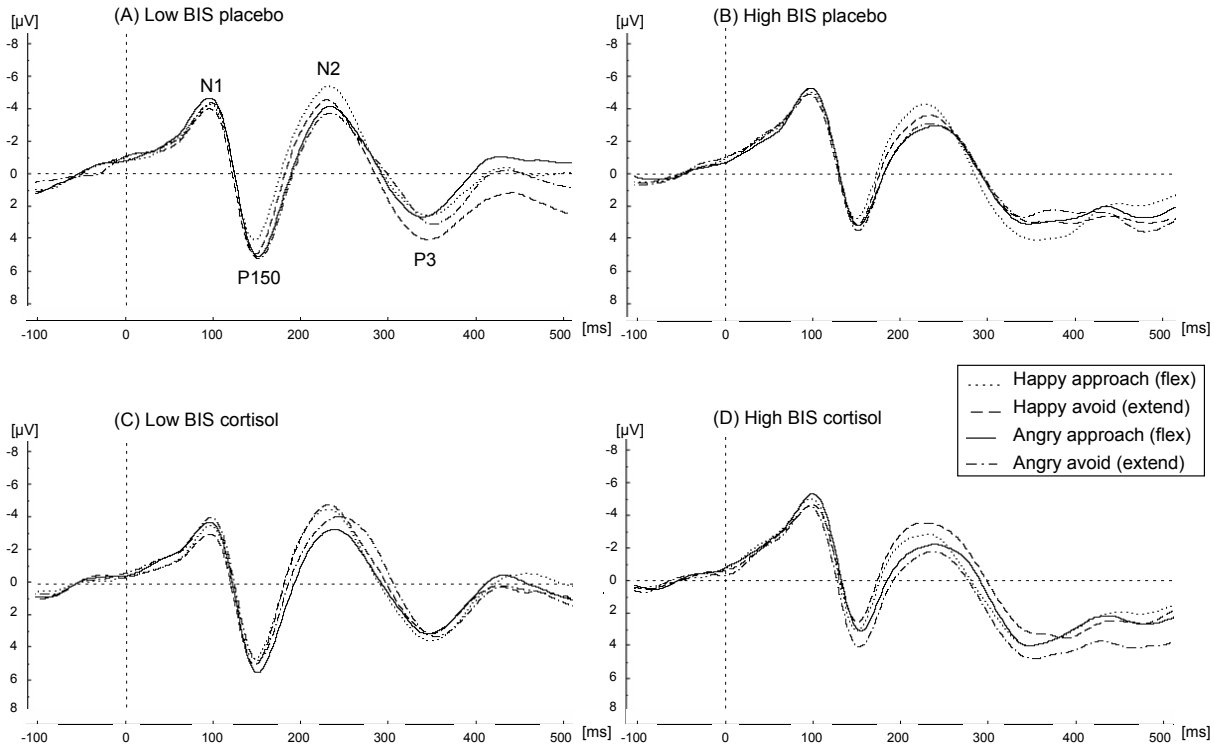

Figure 2.3. Stimulus synchronized event-related potential (ERP) waveforms at Cz for the low BIS (panels A and C) and high BIS (panels B and D) groups after placebo (panels A and B) and cortisol (panels C and D) administration. Stimulus onset was at t = 0. Lines represent the categories defined by the emotional expression of the stimuli and the arm movement of the response.

η^2 = 0.34), but not for happy faces (F(1,19) = 0.68, p = .42). This indicates that only in response to angry faces P150 amplitude was significantly higher (i.e., more positive) when these participants made an avoidant arm movement, than when they made an approaching arm movement. Thus, consistent with the behavioral (IT) results, we found significant congruency effects (i.e., approach versus avoidance) for the high BIS group in the cortisol condition for angry faces only. Also, the P150 amplitude of high BIS participants in the cortisol condition was significantly higher in response to angry compared to happy faces only for avoidant arm movements (emotion: F(1,19) = 14.15, p < .001), and not for approaching arm movements (F(1,19) = 0.00, p = .99).

P3

For P3 amplitude, the group × cortisol × emotion × arm movement ANOVA rm yielded a significant three-way interaction of group × cortisol × arm movement (F(1,36) = 5.13, p < .05, η^2 = 0.13) and a significant three-way interaction of cortisol × emotion × arm movement (F(1,36) = 4.13, p < .05, η^2 = 0.10). Although post hoc analyses of the first interaction did not reveal significant effects, post hoc analyses for the cortisol × emotion

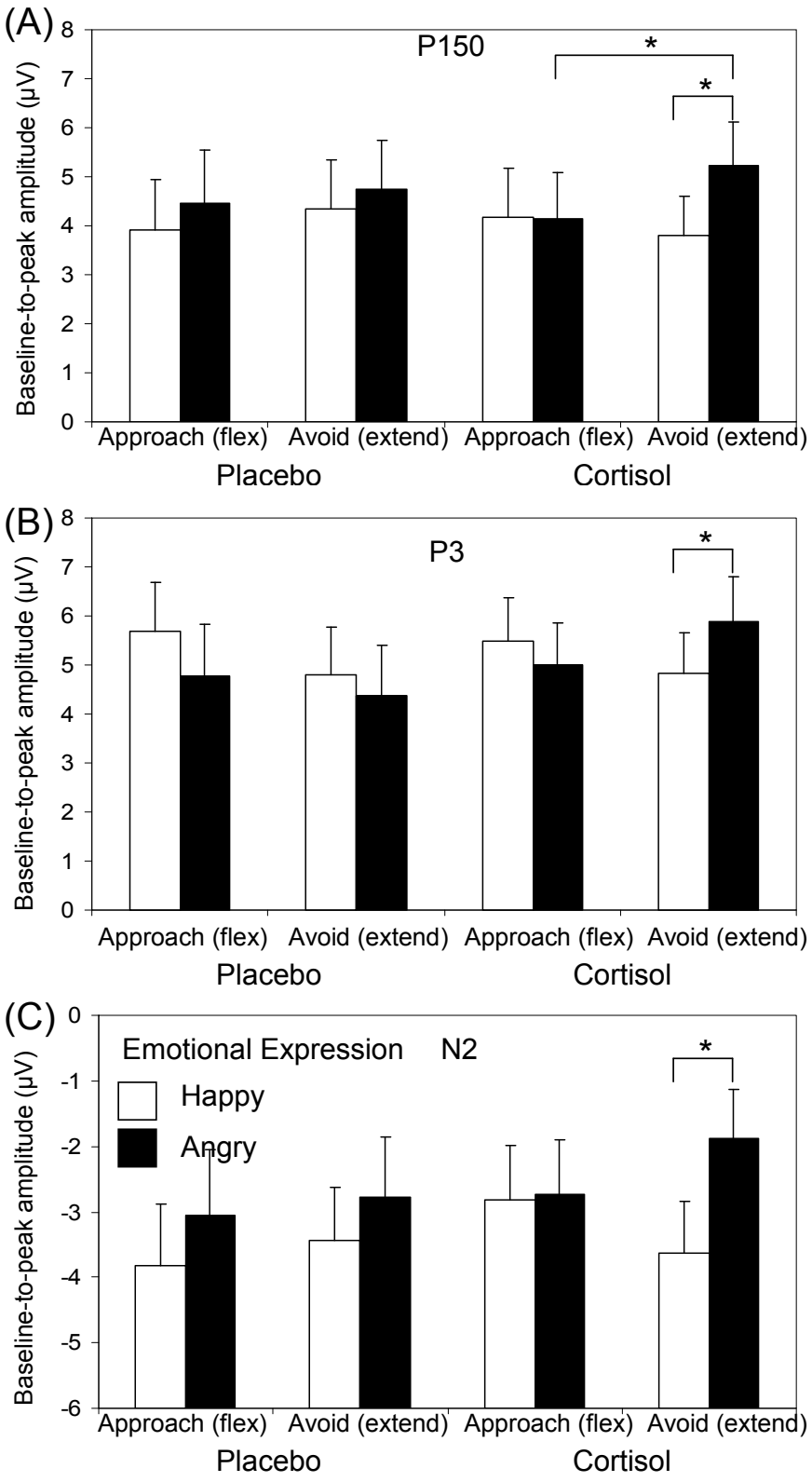

Figure 2.4. Baseline-to-peak amplitude (in μV) for P150 (panel A), P3 (panel B), and N2 (panel C) ERP components for the high BIS group after placebo (left) and cortisol (right) administration. All three components show a significant emotion × arm movement interaction in the high BIS group after cortisol administration only, with most positive amplitudes in response to angry faces when an affect-congruent avoidance response (arm extension) is made. Note that for the N2 component (panel C) the values of the *y*-axis are inverted, such that consistent with panels A and B a higher bar indicates a more positive amplitude. Error bars indicate the standard errors of the means. *$p < .05$.

× arm movement interaction showed the following effects: The emotion × arm movement interaction was significant in the cortisol condition ($F(1,36) = 5.78$, $p < .05$, $\eta^2 = 0.14$), but not in the placebo condition ($F(1,36) = 0.00$, $p = .98$). In the cortisol condition, the P3 amplitude for avoidant arm movements was significantly higher (i.e., more positive) in response to angry faces than in response to happy faces (emotion: $F(1,36) = 8.85$, $p < .01$, $\eta^2 = 0.20$). The four-way *group* × cortisol × emotion × arm movement interaction was not significant ($F(1,36) = 0.02$, $p = .88$), indicating that this effect did not differ between groups. However, because we had specific hypotheses about the congruency effects after cortisol administration in the high BIS participants, we additionally checked whether the emotion × arm movement interaction in the cortisol condition would hold when tested in the high BIS group only. The results indeed indicated that the emotion × arm movement interaction in the cortisol condition was significant for the high BIS group only ($F(1,19) = 4.67$, $p < .05$, $\eta^2 = 0.20$). Interestingly, it was not significant for the low BIS group ($F(1,17) = 1.45$, $p = .25$), nor was it significant for either group in the placebo condition (low BIS: $F(1,17) = 0.57$, $p = .46$; high BIS: $F(1,19) = 0.72$, $p = .41$). Follow up analyses indicated that, in line with the P150 results, for the high BIS group in the cortisol condition the effect of emotion was significant for avoidant arm movements ($F(1,19) = 12.67$, $p < .003$, $\eta^2 = 0.40$), but not for approaching arm movements ($F(1,19) = 0.86$, $p = .37$). Thus, P3 amplitudes were significantly higher in response to angry faces than in response to happy faces only when an avoidant arm movement was made by high BIS individuals in the cortisol condition (see Figure 2.4, panel B).

N1 and N2

We did not find a significant emotion × arm movement interaction for N2 amplitude ($F(1,36) = 0.15$, $p = .70$), indicating that N2 amplitude was not increased for affect-incongruent arm movements (avoidance of happy faces and approach of angry faces) compared to affect-congruent arm movements (approach of happy faces and avoidance of angry faces).

Although we did not have specific expectations about possible effects of cortisol and group on congruency effects for the N2, visual inspection of Figure 2.3 suggested that N2 amplitudes showed similar effects as P150 and P3. This may suggest a general effect of cortisol administration on ERP amplitudes in the high BIS group. Indeed,

significant N2 congruency effects were found in the high BIS group after cortisol administration only (emotion × arm movement: $F(1,19) = 11.55$, $p < .01$, $\eta^2 = 0.38$) (see Figure 2.4, panel C). Thus, although the effects of cortisol administration on the N2 congruency effects were not significant in the four-way ANOVA (group × cortisol × emotion × arm movement: $F(1,36) = 2.38$, $p = .13$), the N2 effects showed trends in the same direction as the IT and positive ERP wave results. As expected we found no other significant effects involving emotion and arm movement on negative waves (N1: all $p > .20$).

Discussion

With the present study we aimed to investigate the influence of cortisol administration on approach and avoidance behavior towards positive and negative social stimuli in high and low avoidant participants (i.e., scoring high or low on the Behavioral Inhibition Scale [BIS]). The second aim was to investigate the associated brain processes using ERPs, with specific focus on components involved in emotional face processing and action monitoring. Compared to low BIS participants, we expected high BIS participants to show relatively increased threat avoidance, and we expected that cortisol administration would result in a facilitation of this threat avoidance. In addition, these behavioral effects were hypothesized to be accompanied by increased amplitudes of ERP components involved in motivational processes. Our results were largely in line with our expectations, showing cortisol administration in high BIS participants to result in enhanced AA congruency effects in both initiation times and positive ERP amplitudes for angry faces. Below, these behavioral and ERP results will be first discussed separately. Thereafter, these results will be integrated in the light of previous findings related to glucocorticoid effects on cognition and threat processing.

Behavioral results

First, consistent with previous findings (Chen & Bargh, 1999; Roelofs et al., 2005; Rotteveel & Phaf, 2004), this study showed the expected congruency effects, as reflected by faster initiation times (IT), faster movement times (MT) and less errors for affect-congruent (i.e., approach happy and avoid angry faces) compared to affect-incongruent (avoid happy and approach angry faces) arm movements.

In addition, in the high BIS, but not the low BIS group, cortisol administration resulted in a significant slowing of approach reactions (IT). In line with our expectations, this resulted in a significant increase of the approach-avoidance congruency effect for angry faces (faster avoidance than approach), but not for happy faces. However, these differential effects for valence should be interpreted with caution. The lack of a four-way interaction including the emotional valence of the faces suggests an inhibition of approach reactions to social stimuli, independent of stimulus valence. However, our present interpretation is supported by the ERP results (as will be discussed later), which do suggest a differential effect of cortisol administration on processing of happy and angry faces.

In spite of the fact that our groups were a priori selected on the basis of extreme high or low BIS scores, and differed significantly with respect to trait anxiety and social anxiety, they did not differ in approach-avoidance reactions in the placebo condition. This may be due to the fact that all participants were healthy students. It is also important to note that basal cortisol levels did not differ between high and low BIS participants. Apparently the approach-avoidance reactions of high and low BIS participants differed only after cortisol administration. These results are consistent with the findings of Roelofs et al. (2005), who also found no differences between high and low trait avoidant individuals (based on a post hoc median-split on BIS scores) on approach-avoidance behavior, independent of cortisol.

Finally, consistent with previous findings (Roelofs et al., 2005), we did not find significant effects of BIS group or cortisol on the error rates or the movement times (MT). This is not surprising, since participants generally make few errors in this task, and MT has been suggested to be predominantly affected by physical parameters of movement, and not by central cognitive processes (see Rotteveel & Phaf, 2004).

ERP results

The second purpose of this study was to investigate the brain processes associated with approach and avoidance of happy and angry faces. In line with the behavioral results, we found a significant effect of cortisol administration on ERPs for high BIS participants only. After cortisol administration P150 amplitude was highest (i.e., most positive) in reaction to angry faces when high BIS participants made an avoidant arm movement. A similar effect was found on P3 amplitude, showing

significantly higher positive amplitudes in reaction to angry as compared to happy faces when an avoidant arm movement was made by high BIS participants after cortisol administration. Although the lack of a four-way interaction including group (low BIS, high BIS) on P3 amplitude indicated that this effect did not differ significantly between groups, separate analyses for each group confirmed our specific a priori expectation that P3 amplitudes in reaction to angry faces would be particularly pronounced for high BIS participants after cortisol administration, given the increased motivational significance of threat stimuli for these participants.

Increased amplitudes of early as well as late positive ERP components have been interpreted as reflecting increased allocation of processing resources to motivationally significant input (Eimer et al., 2003; Nieuwenhuis, Aston-Jones, & Cohen, 2005). The timing of the early effect in the present study (i.e., P150 amplitude) is in line with results of previous studies showing differential processing of faces signaling threat (i.e., fearful or threatening faces, 110-220 ms post-stimulus: e.g., Bar-Haim et al., 2005; Eimer & Holmes, 2002; Schupp et al., 2004; Williams et al., 2006), and suggests an effect on relatively early stages of information processing. Interestingly, data from single-neuron recordings in human ventromedial prefrontal cortex showed differential processing of threatening emotional face stimuli in the same time range (120-170 ms: Kawasaki et al., 2001), suggesting that early aspects of perceptual processing may be modulated via top-down influences, facilitating early identification of, and appropriate behavioral responses to, threat (see Bar, 2003).

The increased P3 amplitude in the present study is consistent with results of other studies indicating increased amplitudes of late positive components for emotionally negative or threat stimuli (e.g., Huang & Luo, 2006; Schupp et al., 2004), which are assumed to reflect more elaborate sustained perceptual processing of relevant emotional stimuli, via top-down influences from limbic and / or frontal areas (Eimer et al., 2003; Krolak-Salmon et al., 2001; Sato et al., 2001). Interestingly, we found enhanced P150 and P3 amplitudes for angry faces after cortisol administration only in high avoidant (high BIS) individuals, indicating that processing of angry faces after cortisol administration was specifically enhanced in individuals sensitive to threat. In addition, here the P150 amplitude for angry faces was significantly higher when an (affect-congruent) avoidance movement was made than when an (affect-incongruent) approach movement was made. This finding may be explained by the fact that the affect-

congruent and affect-incongruent arm movements were blocked in separate instruction conditions, which may have strengthened the response mode within each condition, resulting in priming of affect-congruent stimulus processing.

On a more exploratory basis, we also tested whether the AA task could elicit significant N2 effects, reflected by increased amplitudes for affect-incongruent relative to affect-congruent arm movements. We did not find such effects. In other more frequently used paradigms involving congruent and incongruent stimulus-response mapping, such as Flanker or Stroop tasks, an N2 congruency effect is observed ubiquitously (see e.g., Yeung, Botvinick, & Cohen, 2004). A possible explanation for the discrepancy of our finding is related to the type of conflict that may be elicited by the AA task. In this task, the response conflict in incongruent trials is not the result of two competing endogenous responses elicited simultaneously by the stimulus, as is the case in Flanker and Stroop tasks, but results from a conflict between the instructed response and the intuitive response tendency elicited by the stimulus. Conflict or incompatibility in the AA task may therefore be represented at another level than in typical conflict tasks, and as a result it may not be reflected by increased N2 amplitudes. At present, the representational level at which action or conflict monitoring by the ACC takes place is still unclear (Van Veen, Holroyd, Cohen, Stenger, & Carter, 2004).

Cortisol effects and threat sensitivity

Together, the behavioral and ERP findings showed cortisol administration to be associated with enhanced AA congruency effects in reaction to angry faces in high avoidant, but not low avoidant, individuals. However, whereas this effect was manifested in a slowing of affect-*incongruent* (approach) responses in behavior, ERPs showed enhanced positive amplitudes for affect-*congruent* (avoidance) responses.

The results of this study did not show a general effect of cortisol on approach and avoidance (AA) tendencies. Instead, the effects of cortisol administration on affect-congruent processing of, and initiation times to, angry faces in particular were mediated by individual differences in self-reported threat sensitivity (BIS). This finding may be viewed as consistent with the findings of Roelofs et al. (2005) who investigated the effects of stress-induced cortisol responses on AA behavior, using the same paradigm. In that study, the effects of stress-induction on approach and avoidance tendencies (IT) were found to be mediated by individual differences in cortisol responsiveness, which is

possibly associated with individual differences in the tendency to perceive and respond to affective stimuli (Roelofs et al., 2005).

These results are also in agreement with the findings from animal studies showing that corticosteroid effects on cognition are context dependent, and are influenced by factors such as environmental input and concurrent information processing (De Kloet et al., 1999). People with high BIS scores are suggested to be especially responsive to threat cues (Carver & White, 1994) and thus may have a processing bias for threat-related facial expressions, as has been previously found with anxious individuals (e.g., Bar-Haim et al., 2005; Fox, Russo, & Dutton, 2002; Mogg & Bradley, 2002). This processing bias has been found to increase under stressful conditions (Mathews & MacLeod, 1994), as well as after acute cortisol administration in healthy young males (Putman et al., 2007a). In the present study, the effects of cortisol administration may have interacted with a processing bias of threatening stimuli in high BIS participants. This interpretation is in line with the results of a study by Cools et al. (2005), who found that a manipulation of serotonin function interacted with individual differences in BIS scores to bias the processing of threatening stimuli.

The present study is the first to show effects of cortisol administration on human approach-avoidance behavior, and several related questions remain unanswered. First, it remains to be explored whether the present findings are dose dependent. Second, the effects of cortisol administration in our study do not mimic the behavioral effects of high endogenous cortisol levels during stress, which were found to result in decreased approach and avoidance tendencies (Roelofs et al., 2005). This difference may be explained by the results of several recent studies (e.g., Roozendaal, McReynolds, & McGaugh, 2004; Elzinga & Roelofs, 2005) suggesting that the impairing effects of cortisol on prefrontal functions depend on concurrent noradrenergic activation, which is present during stress, but not in our study. Interesting in this respect is that, consistent with the findings of the present study, Van Honk et al. (1998) found increased (basal) cortisol levels to be associated with increased avoidance of angry faces on an emotional Stroop task, when testing subjects in a non-stress condition. Taken together, these findings suggest an important role for the context in which cortisol levels are elevated. Future studies in which the effects of endogenous cortisol are attenuated, for example with the use of selective steroid receptor antagonists, may help to further assess the role

of cortisol and the interplay with contextual effects in human cognition (De Kloet et al., 1999).

Third, since negative laboratory stimuli are routinely judged to be more arousing than positive laboratory stimuli, the differential effects of cortisol administration on angry faces may be due to either the valence or arousal qualities of these stimuli. We cannot differentiate between these factors in the present study. However, in all likelihood, valence and arousal together influence the motivational significance of these stimuli, to prepare the individual for rapid behavioral responses to stimuli that signal potential danger.

Finally, it should be noted that in order to avoid interactions with hormonal cycling in females, the findings of the present study were based on male participants only, and it remains to be tested whether similar effects emerge for females.

In conclusion, both the behavioral and ERP analyses showed that cortisol enhanced approach-avoidance congruency effects towards angry faces in high avoidant individuals only. ERP analyses showed that amplitudes of both early (P150) and late (P3) positive components were enhanced, suggesting increased processing of threat stimuli after cortisol administration. Together, these results suggest a context-specific effect of cortisol on processing of, and adaptive responses to, motivationally significant threat stimuli, particularly in participants highly sensitive to threat signals. These effects may be relevant for the study of stress and avoidance reactions in patients characterized by strong avoidance tendencies and sensitivity to social threat, such as patients with social anxiety disorder.

Chapter 3

Hypothalamus-Pituitary-Adrenal axis hyperresponsiveness is associated with increased social avoidance behavior in social phobia

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K. Roelofs, J. M. van Peer, E. Berretty, P. de Jong, Ph. Spinhoven, & B. M. Elzinga

Abstract

Background

Social avoidance and inhibition in animals is associated with hyperresponsiveness of the glucocorticoid stress-system. In humans, the relation between glucocorticoid stress-reactivity and social avoidance behavior remains largely unexplored. We investigated whether increased cortisol stress-responsiveness is linked to increased social avoidance behavior in patients with social anxiety disorder (SAD).

Methods

Patients with SAD (n = 18), as well as two control groups of healthy participants (n = 22) and patients with post traumatic stress disorder (PTSD: n = 17), respectively, performed a social approach-avoidance task (AA-task) in a baseline condition and in a social stress condition (provided by the Trier Social Stress Test). The AA-task is a computerized reaction-time task, measuring the speed of manual approach and avoidance responses to visually presented social threat cues (angry faces). Salivary cortisol, blood pressure and subjective anxiety were assessed throughout the experiment.

Results

Patients with SAD showed larger cortisol responses to the social stress test, as compared to healthy and PTSD controls. Most crucially, these increased cortisol responses were significantly correlated to the increase in social avoidance behavior measured by the AA-task in the social stress condition in SAD. An additional regression analysis showed that the cortisol responses predicted the stress-induced increase in social avoidance tendencies over and above the effects of blood pressure and subjective anxiety.

Conclusions

These findings provide the first evidence for a direct link between increased cortisol stress-responsiveness and social avoidance behavior in patients with SAD. The results support animal models of social avoidance and inhibition and may have important treatment implications.

Introduction

Social anxiety disorder (SAD) is the most common anxiety disorder and is characterized by persistent fear and avoidance of social situations (Hofmann & Bögels, 2006; Mannuzza et al., 1995). Avoidance behavior, in particular, plays a crucial role in the maintenance of the disorder and hinders extinction of fear in social situations (Clark & Wells, 1995; Stangier, Heidenreich, & Schermelleh-Engel, 2006). Despite this important role, little is known about the psychobiological mechanisms controlling social avoidance in SAD.

Extensive research in primates has shown that socially avoidant behavior is related to increased activation of the Hypothalamus-Pituitary-Adrenal (HPA)-axis, an important stress system, activation of which results in the release of cortisol (Sapolsky, 1990). For example, primates with relatively high basal and reactive HPA-axis activity show increased social avoidance and behavioral inhibition (Kalin et al., 1998b; Sapolsky, 1990). Also in rats, increased glucocorticoid activity is related to increased behavioral inhibition, particularly in social situations (Cavigelli et al., 2007; Nunez, Ferre, Escorihuela, Tobena, & Fernandez-Teruel, 1996). Human studies have similarly indicated that increased HPA-axis activity in healthy individuals is associated with increased social avoidance and freezing reactions (Roelofs et al., 2005; Roelofs, Bakvis, Hermans, Van Pelt, & Van Honk, 2007; Van Honk et al., 1998, 2000). However, no studies have addressed the relationship between HPA-axis stress-reactivity and social avoidance behavior in patients with SAD and the scarce investigations on cortisol stress-reactivity in these patients have shown mixed results. One study reported increased cortisol responses to a psychological stressor in SAD as compared to healthy controls (Condren et al., 2002), but other investigations failed to find such group difference (Furlan et al., 2001; Levin et al., 1993; Martel et al., 1999). Most importantly, in none of the human studies on SAD were the cortisol responses related to objective measures of overt social fear behavior, such as social approach or avoidance behavior. Such direct comparison is crucial to test the proposed role of hypercortisolism in the failing regulation of social fear and fear behavior in SAD (Hermans & Van Honk, 2006).

The approach-avoidance task (AA-task) (Rotteveel & Phaf, 2004) provides a reliable tool to investigate overt social avoidance behavior and has been shown to be

sensitive to social anxiety and cortisol manipulations in healthy populations (Heuer, Rinck, & Becker, 2007; Roelofs et al., 2005; Van Peer et al., 2007). During this computerized reaction time (RT) task, subjects make approaching or avoiding arm-movements to visually presented pictures of angry facial expressions. Angry faces form a potent threat stimulus in human research and elicit relatively high activation in brain areas involved in threat detection, such as the amygdala, in patients with SAD (Phan et al., 2006; Stein et al., 2002; Straube et al., 2004).

The aim of the present study was to test the effect of stress-induced cortisol on the tendency to avoid angry faces in SAD. To investigate the specificity of this effect, we added happy faces as emotional control stimuli and included healthy participants as well as patients with another anxiety disorder (post traumatic stress disorder, PTSD) as healthy and clinical control groups, respectively. All participants conducted the AA-task in a baseline condition and in a social stress condition, induced by the Trier Social Stress Test (TSST: Kirschbaum et al., 1993). We hypothesized a finding of increased cortisol responses to social stress-induction in patients with SAD, as compared with both healthy and PTSD control subjects. Most crucially, we expected that these increased cortisol responses in SAD are related to an increased tendency to avoid angry faces on the AA-task.

Methods and Materials

Participants

Demographic variables and group characteristics of the three participant-groups are presented in Table 3.1. Patients with generalized SAD and PTSD had been referred for outpatient treatment to a general psychiatric hospital and were diagnosed with the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-i/p: First, Spitzer, Gibbon, & Williams, 1996). Only PTSD patients with a history of interpersonal trauma (physical and/or sexual abuse) were included, because they are also characterized by marked fear and avoidance of social situations. Healthy control (HC) participants were recruited via advertisements in local newspapers. Exclusion criteria for participation were: age < 18 or > 65 years, left-handedness, psychotic disorder, use of neuroleptics, substance use/addiction, a chronic disease, use of corticosteroids max 6 months before participation, and pregnancy or breast feeding. Of the 22 SAD patients originally tested,

Table 3.1. Demographic Variables and Group Characteristics

Variable	SAD *n* = 18	PTSD *n* = 17	HC *n* = 22	*F* value	*p*
Gender (*n*: male/female)	9M/9F	6M/11F	9M/13F	0.8 (X^2)	*ns*
Age (yrs)	32.2 (± 3.2)	35.8 (± 3.3)	39.9 (± 2.9)	1.5	*Ns*
Medication [a]	7N/11Y	4N/13Y	17N/5Y	12.2 (X^2)	.002
Antidepressants	6	4	0		
Sedatives[b]	4	2	0		
Other[c]	1	3	2		
Oral contraceptives	4	6	4		
Social anxiety (SPAI)	156 (± 7.7)[d]	131 (± 7.9)[d]	89 (± 7.0)[d]	21.9	<.001
Depression (BDI)	16.7 (± 2.5)[e]	17.9 (± 2.6)[f]	3.7 (± 2.3)[ef]	10.9	<.001
Early trauma (TEQ)	3.6 (± 0.8)	7.4 (± 1.0)[g]	2.7 (± 0.8)[g]	7.9	<.001
Emotional	1.6 (n = 8)[h]	1.5 (n = 6)	0.5 (n = 5)[h]	2.0	.1
Physical	0.6 (n = 3)	1.9 (n = 9)[i]	0.5 (n = 4)[i]	4.2	.020
Sexual	0.2 (n = 1)	2.0 (n = 9)[j]	0.1 (n = 1)[j]	12.9	<.001
TSST-modifications[k] (*n*)	11N/7Y	11N/6Y	21N/1Y	6.8(X^2)	.033
Verbal encouragements	1	3	1		
Structured speech	1	0	0		
Speech terminated preterm	5	3	0		
Axis-1 comorbidity[l] (*n*)					
Generalized anxiety d.	2	2	0		
Obsessive compulsive d.	1	2	0		
Panic d.	0	3	0		
Depressive d.	7	4	0		
Dysthymic d.	1	1	0		

Data from questionnaires are presented in mean score and SEM. SAD = social anxiety disorder; PTSD = posttraumatic stress disorder; HC =healthy control subjects; N = no; Y = yes; BDI =Beck Depression Inventory; SPAI = Social Phobia and Anxiety Inventory; TEQ = Traumatic Experiences Questionnaire; d = disorder.

[a]Total numbers do not correspond to the sum of the four subtypes, because of overlap (patients might use more than one type of medication).

[b]Participants refrained from taking the drug minimally 24 hours before the experiment.

[c]Including medication for respiratory tracts and blood pressure control.

[d–j]Cells with similar superscripts differ significantly from each other (p<.05).

[k]See "Procedures" section for a detailed description of the Trier Social Stress Test (TSST) modifications.

[l]Assessed with the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-i/p).

4 patients had to be excluded (due to excessive blood pressure, *n* = 1; missing AA-task data due to technical problem, *n* = 2; and immediate termination after introduction TSST, *n* = 1), leaving 18 SAD patients for the analyses. Of the 20 PTSD patients tested, 3 were excluded (heart rhythm disturbances, *n* = 1; technical problems, *n* = 2), leaving a total of 17 PTSD patients. None of the 22 HC participants were excluded.

Before the test day, all participants were instructed to minimize physical exercise

and not to smoke during the hour preceding the experiment and not to take large meals, coffee, and drinks with low pH during the night (from 8:00 PM on) and morning preceding the test day, because these variables can affect cortisol levels. All participants agreed on this procedure, and nobody reported difficulty with abstaining from caffeine or nicotine. All participants had normal or corrected-to-normal vision and provided written informed consent. The study was approved by the local ethics committee.

AA-task

In this affect-evaluation task (Rotteveel & Phaf, 2004), participants respond to visually presented pictures displaying emotional facial expressions, by making arm movements towards (arm-flexion or approach) or away from (arm-extension or avoid) their own body. Eighty pictures with facial expressions (Ekman & Friesen, 1976; Lundqvist et al., 1998; Martinez & Benavente, 1998; Matsumoto & Ekman, 1988) served as stimuli. Both the happy and angry expressions were taken from the same model (total of 40 models [50% male]). The stimuli were subdivided into four fixed series (A1-A2-B1-B2) with each 10 happy and 10 angry expressions from different models. Each picture was presented on a 15-inch computer screen with a vertical visual angle of 14° and a horizontal visual angle of 10.7°. The approach and avoidance responses were given by means of three one-button boxes that were fixed to a vertical stand (see Rotteveel and Phaf, 2004, for a photograph of this experimental set-up). Participants were seated to the left of the stand allowing them to respond with their right hand. For the resting position of the right hand participants were instructed to push the home-button (fixed in the middle) loosely with the back of the right hand as long as no response was given, ensuring that the movement distance for approach and avoidance responses would be equal. All subjects received an affect-congruent and an affect-incongruent instruction-block of trials, both before (A1-A2) and after (B1-B2) stress-induction. In affect-congruent instruction-blocks, participants were instructed to press the upper button (approach movement) in response to a happy face and to press the lower button in response to an angry face (avoidance movement). Affect-incongruent instruction blocks involved the opposite stimulus-response mappings (approach-angry, avoid-happy). No reference was made in the instructions to congruence and incongruence, approach and avoidance, or arm flexion and extension. The order of instructions before and after stress-induction was fully counterbalanced across subjects.

Each instruction-block was preceded by 12 practice trials containing pictures not included in the experiment. Trials started with a 100-msec-lasting black fixation point at the center of the screen. After an interval of 300 msec (blank screen), the face-stimulus was presented for 100 msec, with an inter-trial-interval (ITI) of 1500 msec. The RT (time between stimulus-onset and response) was measured with an accuracy of < 2 msec.

The Trier Social Stress Test

This psychological challenge test consists of a free speech (5 min) and a mental arithmetic task (5 min), performed in front of an audience of 3 individuals and preceded by an anticipation phase (5 min), in which participants prepare the public speech. During the speech participants take on the role of a job applicant (job definition is predefined, based on what is challenging and relevant for the participant; see Kirschbaum et al., 1993, for exact description). This 15-min protocol has been found repeatedly to induce significant endocrine and cardiovascular responses in approximately 70 % of the participants (Kirschbaum et al., 1993). In case participants reported being unable to proceed with the free speech, they received a modified version of the TSST, with up to three measures helping participants to fulfill the task, without dropping out. The measures were in fixed order and the next step was only applied if necessary: 1) giving verbal encouragements during the free speech; 2) structured interview instead of free speech, involving direct questions about job application; and 3) preterm cancellation of free speech and immediate continuation with the mental arithmetic task. The modifications applied in each group are indicated in Table 3.1.

Physiological and subjective measures

Salivary cortisol, blood pressure, and subjective anxiety were measured to assess glucocorticoid, autonomic, and subjective stress-responses, respectively. All three stress-measures were obtained at 10 assessment points over a 130-min period at -65, -35, -25, -10, 0, +15, +25, + 40 + 50 and +65 min, respectively, with reference to the start of the stressor (see Figure3.1). All assessments were performed between 1:00 PM and 4:00 PM.

To assess free cortisol levels, saliva samples were obtained with Salivette collection devices (Sarstedt, Rommelsdorf, Germany). The samples were stored at -20°C

before assaying. Biochemical analysis of free cortisol in saliva was performed using a competitive electrochemiluminescence immunoassay (ECLIA; Elecsys 2010, Roche Diagnostics, Basel, Switserland), as described elsewhere (Van Aken et al., 2003). Blood pressure (diastolic) was measured from the nondominant arm with an automatic blood pressure monitor (Omron R5-I) that could be initiated manually. Subjective anxiety was rated on a 10-cm visual analoge scale ranging from "not anxious" to "very anxious".

Questionnaires

The social anxiety subscale of the Social Phobia and Anxiety Inventory (SPAI: Bögels & Reith, 1999; Turner et al., 1989) is a 32 item self-report scale measuring social anxiety. Scores range from 0 to 192. The Beck Depression Inventory (BDI-II: Beck, Ward, Mendelsohn, Mock, & Erbaugh, 1961; Bouman, Luteijn, Albersnagel, & Van der Ploeg, 1985) is a 21-item self-report questionnaire that measures depressive symptoms during the past week. Scores range from 0 to 63. The Traumatic Experiences Checklist (TEC: Nijenhuis, Van der Hart, & Kruger, 2002) is a 26-item self-report inventory assessing emotional, physical and sexual abuse. The total score ranges from 0 to 26, the emotional abuse score from 0 to 6, the physical abuse score from 0 to 5, and the sexual abuse score from 0 to 6.

Procedure

All participants visited the lab twice. On the first (assessment) day, a trained psychologist administered the SCID-i/p (25) and the questionnaires (see Table 3.1). On the second (test) day, participants arrived at the laboratory at 1:00 PM. After the first series of physiological and subjective assessments, subjects were adjusted to the experimental environment with a relaxing movie. The baseline administration of the AA-task took place after the third assessment (-25 min with reference to the onset of the stressor; Figure 3.1). After two additional cognitive tests (an attention and a learning test, of which results will be reported elsewere) the experimenter introduced the TSST that was administered by three other experimenters (audience). After the TSST, the audience called the experimenter back into the room and requested him to administer the cognitive tests, including the AA-task, again in their presence (+ 25 min with reference to onset TSST). In this way, the social evaluation context remained present during the second administration of the AA-task. Subsequently, the audience left the

room and returned for a debriefing after the last physiological assessment had taken place. One week after the experiment patients were contacted for a second debriefing.

Statistical analyses

Reaction time outliers were filtered with a <150- and >1000 msec cut-off. For each participant, the median of the remaining RTs (97%) for the correct responses were calculated/ cell (defined by Condition [baseline, stress], Valence [happy, angry face], and Movement [approach, avoid]). The influence of stress-induction on the stress measures and the task performance was tested with repeated measures Analyses of Variance (ANOVA-rm). Because gender and use of medication can influence the cortisol stress-response (Kirschbaum et al., 1993) and because groups differed with respect to the TSST-modifications, we controlled for these variables in a separate analysis by including gender, medication and TSST-modifications as covariates (ANCOVA).

The total cortisol response for each participant was computed with the area under the curve with reference to the ground (AUCg: see Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003 - formula 2). The effect of cortisol responses on the task performance in SAD was analyzed by entering the individual cortisol responses (AUCg) as a continuous factor into the analyses (analysis of covariance: see Judd, Kenny, & McClelland, 2001). Significant interactions including this factor were followed up by calculating Pearson correlations between this factor and the within-subject effects. The specificity of the effect of cortisol among other stress measures was tested using an additional linear regression analysis with cortisol, blood pressure and subjective anxiety (all in AUCg) as predictors and the "stress-induced change in task performance" as dependent variable. For all analyses alpha was .05. Effect-sizes of significant results are reported with the Partial Eta Squared (η^2). Within-subject effects are reported with Greenhouse-Geisser correction.

Results

Stress measures

Separate two-way ANOVAs rm were conducted for each of the three stress-measures (cortisol, blood pressure, and subjective anxiety) with within-subject factor Time (10 assessment points) and between-subject factor Group.

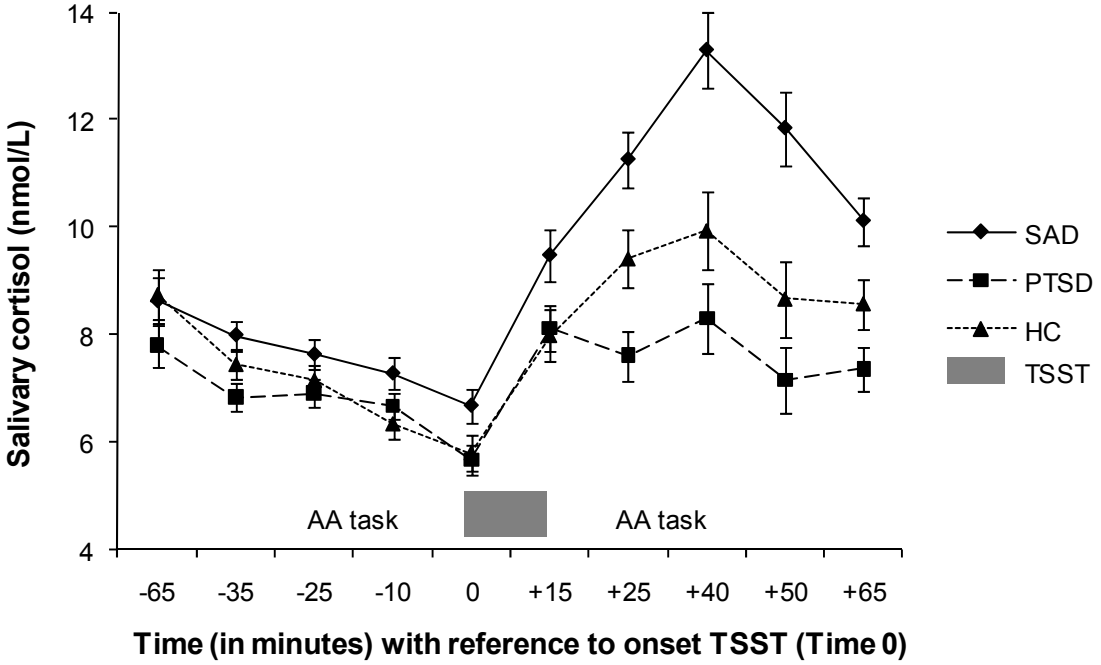

Figure 3.1. Cortisol responses before and after stress exposure the Trier Social Stress Test (TSST). Patients with social anxiety disorder (SAD) have larger cortisol responses to the TSST than both control groups of healthy subjects (HC) and patients with posttraumatic stress disorder (PTSD). AA, approach-avoidance.

Cortisol

Analysis of the three subject-groups together yielded a trend toward a main effect of Group ($F(2,54)=2.54$, $p = 0.08$, $\eta^2 = 0.09$; see Figure 3.1). This effect remained present when controlling for gender, medication and TSST-modifications, by adding these variables as covariates to the analysis ($F(2,51) = 3.18$, $p = 0.050$, $\eta^2 = 0.11$). The TSST-modification was the only covariate showing a significant main effect ($F(1,51) = 4.51$, $p = 0.039$, $\eta^2 = 0.08$). Post-hoc "two-Group" comparisons (while controlling for the effects of TSST-modification) showed significant Group-effects for "SAD-versus-HC" ($F(1,37)= 4.61$, $p = 0.038$, $\eta^2 = 0.11$) and for "SAD-versus-PTSD" ($F(1,32) = 4.56$, $p = 0.041$, $\eta^2 = 0.13$), but not for the "HC-versus-PTSD" comparison ($F(1,36) = 0.12$, *ns*). Most crucially, a Group × Time interaction emerged for both "SAD-versus-HC" ($F(2,60) = 4.44$, $p = 0.022$, $\eta^2 = 0.11$) and "SAD-versus-PTSD" ($F(2,53) = 3.34$, $p = 0.050$, $\eta^2 = 0.09$) but not for the "HC-versus-PTSD " comparison ($F(3,98) = 0.86$, *ns*). These Group × Time interactions were explained by the fact that SAD patients had larger cortisol responses than both control groups after stress-induction (assessment 6-10; SAD-HC: $F(1,37) = 4.36$, $p = 0.044$, $\eta^2 = 0.11$); SAD-PTSD: $F(1,32) = 4.75$, $p = 0.037$, $\eta^2 = 0.13$) but not at baseline

(assessment 1-5: all p >0.35) (Figure 3.1). Further analyses to reveal the effects of TSST-modifications in the SAD group, demonstrated that SAD patients who received TSST-modifcations (n = 7) had significantly lower cortisol responses as compared with those who underwent the original TSST (n = 11: $F(1,21) = 4.10$, $p = 0.05$, $\eta^2 = 0.21$). For this reason, the SAD-versus-HC comparison yielded a significant Group × Time interaction, either when controlling for TSST-modifications (see preceding text) or when excluding participants that received TSST-modifications ($F(2,45) = 4.38$, $p = 0.029$, $\eta^2 = 0.13$) but not when TSST-modifications were not accounted for ($F(2,57) = 1.22$, *ns*). Similarly, the Group × Time interaction for the SAD-versus-PTSD comparison remained significant, when the subjects with TSST-modifications were excluded ($F(2,34) = 3.40$, $p = 0.05$, $\eta^2 = 0.15$) but not when TSST-modifications were not controlled for ($F(2,52) = 2.45$, *ns*).

Diastolic blood pressure

A significant main effect of Time ($F(7,352) = 13.22$, $p < 0.001$, $\eta^2 = 0.20$) indicated increased blood pressure after stress-induction as compared with baseline. There were no effects involving Group (all $p > 0.2$).

Subjective anxiety

There were significant main effects of Time ($F(5,267) = 44.05$, $p < 0.001$, $\eta^2 = 0.45$) and Group ($F(2,54) = 12.91$, $p < 0.001$, $\eta^2 = 0.32$). These effects remained present when controlling for Gender, Medication and TSST-modification by entering these variables as covariates into the analysis (all $p < 0.05$). None of the covariates yielded a significant effect (all $p > 0.3$). Post-hoc "two-Group" comparisons indicated that both patient groups scored higher than healthy controls ("SAD-versus-HC": $F(1,38) = 26.39$, $p < 0.001$, $\eta^2 = 0.41$; "PTSD-versus-HC": $F(1,37) = 18.67$, $p < 0.001$, $\eta^2 = 0.34$), but did not differ mutually ("SAD-versus-PTSD": $F(1,36) = 0.46$, *ns*).

In sum, stress-induction was successful, as evidenced by significant Time-effects on all stress measures. Most importantly, in agreement with our first hypothesis, SAD patients showed significantly larger stress-induced cortisol levels, as compared with both healthy and PTSD controls. In contrast, stress-induction did not elicit (additional) differential group effects for blood pressure and subjective anxiety.

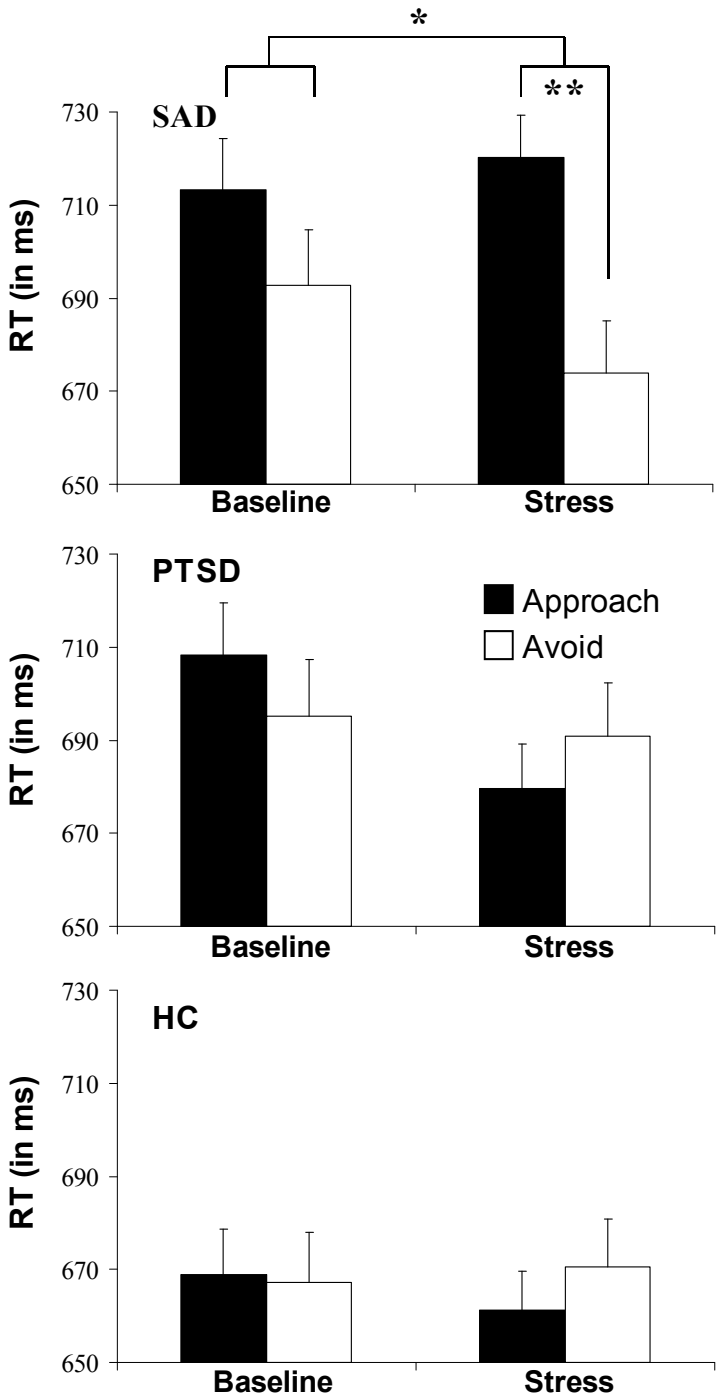

Figure 3.2. Reaction times (RT; in msec) for approach and avoidance movements towards angry faces on the AA-task in patients with SAD, patients with PTSD and HC subjects. A significant Condition (Baseline, Stress) × Movement (Approach, Avoid) interaction for SAD patients indicated that social stress induction resulted in increased AA-effects for angry faces. Groups only differed during stress and not at baseline, indicating larger AA-effects for angry faces in SAD, as compared with PTSD and HC. Abbreviations as in Figure 1. *p<.05; **p<.01

Effects of stress-induced cortisol on threat avoidance in SAD

To test our second hypothesis (stress-induced cortisol predicts avoidance of angry faces on the AA-task in SAD) we conducted a two-way ANOVA-rm for the RTs for

Table 3.2. Mean RTs and ERs for approach and avoidance responses to angry and happy faces on the AA-task for each group in baseline and stress conditions

	Baseline						Stress					
	SAD		PTSD		HC		SAD		PTSD		HC	
	app	av	app	av	app	av	app	av	app	av	app	av
Angry Face Responses												
RT	713 (22)	693 (24)	708 (22)	695 (24)	669 (20)	667 (21)	720 (18)	674 (22)	680 (19)	700 (23)	661 (17)	671 (20)
ER	6.2 (2.4)	3.9 (2.1)	6.7 (2.4)	7.5(2.1)	9.6 (2.2)	9.4 (1.9)	6.5 (1.8)	7.2(2.5)	7.4 (1.8)	9.5 (2.5)	5.3 (1.6)	4.2 (2.2)
Happy Face Responses												
RT	668 (25)	701 (30)	683 (26)	702 (31)	632 (23)	683 (27)	680 (26)	697 (26)	696 (26)	684 (27)	635 (23)	685 (24)
ER	4.4 (1.6)	2.6 (1.7)	6.6 (1.6)	5.8 (1.7)	7.6 (1.4)	4.1 (1.5)	6.6 (1.4)	4.6 (1.7)	4.9 (1.4)	5.9 (1.7)	4.3 (1.3)	2.8 (1.6)

Values presented as mean(SEM). RT = reaction time in msec; ER = error rates in %; app = approach; av = avoid; other abbreviations as in Table 3.1.

angry faces, with Condition (baseline, stress) and Movement (approach, avoid) as within-subject factors and the individual cortisol levels (AUCg) as a continuous factor. The results showed a significant Condition × Movement effect ($F(1,16) = 5.49$, $p = 0.032$, $\eta^2 = 0.26$), reflecting a significant effect of Movement during stress ($F(1,16) = 8.63$, $p = 0.009$, $\eta^2 = 0.34$) but not at baseline ($F(1,16) = 0.64$, *ns*) in SAD. These findings indicate that SAD patient are faster in avoiding than approaching angry faces (AA-effect) in the social stress condition (see Figure 3.2 and Table 3.2). Most importantly, a significant Condition × Movement × Cortisol interaction ($F(1,16) = 6.55$, $p = 0.021$, $\eta^2 = 0.29$) indicated that the patients' cortisol levels correlated significantly with the stress-induced increase in AA-effects. Pearson correlations between the cortisol levels and the stress-induced change in AA-effects (Δ AA-effects) showed that the direction of this correlation was positive ($r = 0.54$, $p = 0.02$; see Figure 3.3A), indicating that high cortisol levels are associated with an increased tendency to avoid social threat in SAD. There was no such relation for the HC subjects ($r = -0.06$, $p = 0.79$; Figure 3.3B) or for the PTSD control subjects ($r = 0.13$, $p = 0.63$). To test whether the Condition × Movement effect in SAD remained present when controlling for Gender, Medication and TSST-modifications, these variables were entered as covariates in the two-way ANOVA-rm. Both the Condition × Movement ($F(1,13) = 4.60$, $p = 0.05$, $\eta^2 = 0.26$) and the Condition × Movement × Cortisol ($F(1,13) = 5.17$, $p = 0.041$, $\eta^2 = 0.29$) interactions remained significant and there were no main effects for the covariates (all $p > 0.2$). In addition, we

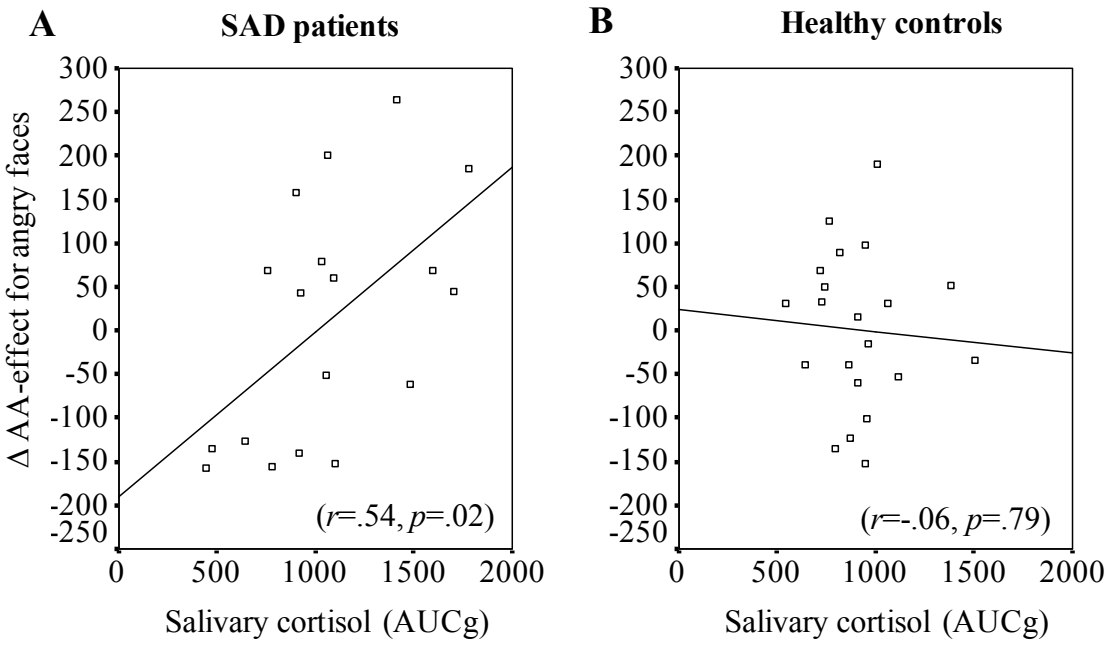

Figure 3.3. Correlation between cortisol levels (area under the curve [AUCg]) and stress-induced change in approach-avoidance effect (Δ AA-effect) for angry faces in patients with social anxiety disorder (SAD) (A) and healthy control (HC) subjects (B). The scatter plot in A shows that high cortisol levels are associated with increased AA-effects after stress induction, indicating that high glucocorticoid stress-responsiveness in SAD is associated with a stress-induced increase in the tendency to avoidance angry faces. There was no such effect in HC subjects (B). AA-effect, RT approach - RT avoid; Δ AA-effect = (["RT approach - RT avoid" in stress] – ["RT approach minus RT avoid" at baseline]). Positive Δ AA-effects indicate larger AA-effects after stress, as compared with baseline. RT, reaction time.

controlled for depression rates by entering the individual BDI-II total-scores as a covariate in the two-way ANOVA-rm. Both the Condition × Movement ($F(1,15)= 9.50$, $p = 0.008$, $\eta^2 = 0.39$) and the Condition × Movement × Cortisol ($F(1,15) = 7.45$, $p = 0.015$, $\eta^2 = 0.36$) interactions remained significant and there was no main effect for the depression-rates ($F(1,15) =0.74$, *ns*).

Finally, to test whether cortisol predicted the stress-induced change in AA-effects over and above the effects of blood pressure and subjective anxiety, the relative contribution of these three stress measures (all in AUCg) on the Δ AA-effects were tested in a regression analysis. The total model explained a statistically significant part of 68% of the variance of the Δ AA-effects ($R^2 = 0.46$, $F(3,16) = 3.65$, $p = 0.042$) and cortisol was the only significant predictor (see Table 3.3).

Together these findings support our second hypothesis that stress-induced cortisol is related to an increase in social avoidance in patients with SAD.

In both control groups, the same Condition × Movement ANOVA-rm for the angry face responses did not yield significant effects (all $p > 0.4$; see Figure 3.2). Visual

Table 3.3. Regression analysis predicting the stress-induced increase in approach-avoidance effects for angry faces (Δ AA-effect) in patients with SAD.

Predictor	*Beta*	*T*	*P*
(Constant)		-1.46	*ns*
Cortisol (AUCg)	0.68	2.65	0.02
Diastolic blood pressure (AUCg)	0.11	0.46	*ns*
Subjective anxiety (AUCg)	0.40	1.74	*ns*

Total model: R^2= 0.46 (F(3,16) = 3.65, p= 0.042)

Δ AA-effect, approach-avoidance effects for angry faces; SAD, social anxiety disorder; AUCg, area under the curve with reference to the ground.

inspection of Figure 3.2 suggests a group difference in AA-effects in the stress-condition. We tested this additional hypothesis for the stress-condition with a two-way ANOVA-rm with within subject-factor Movement and between-subject factor Group and indeed found a significant Group × Movement interaction in the stress-condition (F(2,54) = 3.71, p = 0.031, η^2 = 0.12), indicating that the earlier reported Movement-effect for SAD was not present for HC and PTSD groups (all p > 0.2). There was no such group difference at baseline (F(2,54) = 0.23, *ns*).

There were no effects involving Condition or Group for the happy face responses, nor for the error rates (all p > 0.1; see Table 3.2).

Discussion

The purpose of the present study was to investigate the effects of stress-induced cortisol on social avoidance behavior in patients with social anxiety disorder (SAD). Three major findings emerged from the present study. First, patients with SAD, as compared with both healthy and PTSD control subjects, showed increased cortisol responses to social stress-induction. Second, social stress elicited increased avoidance tendencies toward social threat stimuli in SAD. Most crucially, the cortisol responses predicted the increase in social avoidance tendencies during stress in patients with SAD. This effect remained after controlling for possible confounders such as gender and medication. Moreover, cortisol predicted social avoidance behavior over and above the effects of blood pressure and subjective anxiety.

Our findings of increased cortisol stress-reactivity in SAD confirm findings by Condren et al. (2002), indicating increased (plasma) cortisol in response to a mental arithmetic/working memory task in SAD. The results contrast with findings by Martel et

al. (1999) and Levin et al. (1993) who applied a public speaking task in adolescent and adult SAD patients, respectively. Also Furlan et al. (2001) found no differential cortisol responses comparing SAD patients and HC subjects when speaking in front of a camera (although comparison of only the cortisol-responders within each group yielded larger cortisol levels for SAD-responders). One possible explanation for the nonsignificant findings in most of the previous studies is that patients with SAD tend to show less compliance to the stress-protocol than HC. None of the previous studies on social evaluation-stress in SAD provided information on protocol-compliance or drop-out. However, 7 of our 18 SAD patients reported being unable to proceed with the TSST during the public speaking task. To prevent these patients from dropping out, we formalized up to three mild modifications (e.g., verbal encouragements, structured interview, or preterm termination of the free speech). These patients who initially expressed the highest distress during the task showed significantly lower cortisol responses than those who completed the task without modifications. As a result, some group differences in cortisol response only emerged when controlling for these modifications. This finding indicates that even mild protocol violations may critically reduce the cortisol responses and should be taken into account in future studies.

SAD patients showed significant avoidance tendencies to angry faces on the AA-task (see also Heuer et al., 2007). This effect was particularly pronounced in a condition of social evaluation-stress (Mansell, Clark, Ehlers, & Chen, 1999; Roelofs et al., 2005), in line with previous findings in highly anxious subjects,. Most importantly, the present study is the first to show a relation between an objective measure of social avoidance behavior and cortisol stress-responses in patients with SAD. Stress-induced increases in avoidance tendencies were positively correlated to the cortisol responses. This finding shows an interesting parallel with primate studies demonstrating social fear behavior, such as social avoidance and freezing, to be associated with increased cortisol stress-reactivity (Kalin et al., 1998b; Sapolsky, 1990). The findings also fit with findings by van Peer et al. (2007) showing that cortisol administration resulted in increased AA-effects in high (and not low) socially anxious subjects. Cortisol also increased the processing of the angry faces in these subjects only, as evidenced by increased P2 amplitudes in the electroencephalogram (EEG) for angry-avoid trials, which may indicate that cortisol facilitates threat avoidance in high anxious individuals. This interpretation fits theories suggesting that cortisol plays an important role in the regulation of the stress-response

and the selection of behavioral responses such as an avoidance response (Condren et al., 2002; De Kloet et al., 1999; Sapolsky, 1990) and might explain our findings of faster avoidance as compared to approach responses during stress. Alternatively, the relatively larger RT costs for angry-approach trials in the social stress-context may result from the direct effects of cortisol on prefrontal inhibitory functioning. Affect-incongruent angry-approach trials require the inhibition of an automatic response-tendency (avoid angry face) and the selection of a counter-intuitive response, and both are frontal functions as indicated by a recent fMRI-study using the same AA-task (Roelofs et al., 2009b). Several studies have demonstrated that stress-levels cortisol can impair frontal (often stress-irrelevant) functions such as working memory and inhibitory control (e.g., Lyons, Lopez, Yang, & Schatzberg, 2000; Skosnik, Chatterton, Swisher, & Park, 2000). A similar mechanism may also have caused the high RT costs for the affect-incongruent angry-approach trials in SAD.

A strength of the present study is that stress-induced cortisol was investigated in direct relation with social avoidance performance in SAD. Also, possible TSST-modifications were fully formalized. A limitation is the fact that only free cortisol was measured. However, the methods to assess other HPA-axis measures (such as corticotropin-releasing hormone) are invasive and of potential threat for requiring good (stress-free) baseline assessments (Kirschbaum et al., 2003; Kirschbaum & Hellhammer, 1994). The effect sizes for the cortisol-responses were relatively small and the fact that we found no significant cortisol and avoidance responses in PTSD controls may be related to the social nature of the TSST and the AA-task. Additional research in other anxiety disorders using disorder-specific materials is needed to identify the specificity of the effects (Harvey, Watkins, Mansell, & Shaffran, 2004). Also, although cortisol predicted a significant part of the change in approach-avoidance tendencies in SAD patients, other elements of the social stress condition might have contributed to this effect as well. Further research is needed to disentangle the relative contribution of, for example, the presence of the audience during the second administration of the AA-task versus the preceding stressor. In addition, possible group differences in sensitivity to repeated administration of the AA-task might have confounded the results and should be accounted for in future studies. Finally, we should note that the relationship between cortisol and social avoidance is correlational in nature, allowing no causal interpretations.

To conclude, the present findings for the first time show that high glucocorticoid stress-responses in SAD are associated with increased social avoidance tendencies during social exposure. Avoidance behavior plays a crucial maintaining role in SAD and forms a major target for clinical interventions, such as exposure-therapy. Our data suggest that high levels of endogenous cortisol during social exposure may hinder the execution of threat-approach behavior and may increase threat-avoidance behavior. An important implication may be that the level of stress (and stress-induced cortisol, in particular) during exposure therapy should be kept to a limit in order to achieve optimal benefits from the therapy. These findings are in line with the systematic desensitization approach originally formulated by Wolpe (1958) requiring the therapist to initiate only small amounts of distress in the patient. As a step further along these lines, individual glucocorticoid stress-sensitivity might be assessed to tailor and fine tune psychological and pharmacological interventions in patients with SAD.

Chapter 4

Cortisol-induced enhancement of emotional face processing in social phobia depends on symptom severity and motivational context

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J. M. van Peer, Ph. Spinhoven, J. G. van Dijk, & K. Roelofs

Abstract

We investigated the effects of cortisol administration on approach and avoidance tendencies in 20 patients with social anxiety disorder (SAD). Event-related brain potentials (ERPs) were measured during a reaction time task, in which patients evaluated the emotional expression of photographs of happy and angry faces by making an approaching (flexion) or avoiding (extension) arm movement. Patients showed significant avoidance tendencies for angry but not for happy faces, both in the placebo and cortisol condition. Moreover, ERP analyses showed a significant interaction of condition by severity of social anxiety on early positive (P150) amplitudes during avoidance compared to approach, indicating that cortisol increases early processing of social stimuli (in particular angry faces) during avoidance. This result replicates previous findings from a non-clinical sample of high anxious individuals and demonstrates their relevance for clinical SAD. Apparently the cortisol-induced increase in processing of angry faces in SAD depends on symptom severity and motivational context.

Introduction

In a recent study, we investigated the effects of cortisol administration on threat processing and approach and avoidance behavior in a non-clinical sample of high and low anxious students (Van Peer et al., 2007). The results of that study showed relatively faster avoidance behavior as well as enhanced positive amplitudes (P150 and P300) on midline electrodes during avoidance of angry faces after cortisol administration, indicating increased processing of threat stimuli during threat avoidance. Importantly, these effects were found only in high and not low anxious participants, suggesting a context-specific effect of cortisol on threat processing in participants highly sensitive to threat signals. These findings may be very relevant for patients characterized by strong avoidance tendencies and sensitivity to social threat, in particular patients with social anxiety disorder (SAD). Therefore, with the present study we aimed to replicate and extend these findings in a clinical group of patients with generalized SAD.

The stress hormone cortisol (corticosterone in animals) plays an important role in the regulation of social motivational behavior (e.g., Kalin et al., 1998a; Roelofs et al., 2005, 2007, 2009a; Sapolsky et al., 2000; Van Honk et al., 1998, 2000; Van Peer et al., 2007). In addition, dysregulation of cortisol levels is implicated in the development and maintenance of various mood and anxiety disorders (e.g., Roelofs et al., 2009a; De Kloet, et al., 2005; Holsboer & Ising, 2008). However, studies investigating the effects of cortisol on cognitive-emotional processes have focused heavily on declarative memory (see Lupien et al., 2007 for a comprehensive review) and studies examining cortisol effects on threat processing and avoidance behavior in humans are scarce. Nevertheless, the results of some recent studies in healthy human subjects show that cortisol can affect threat processing and avoidance behavior, especially in high anxious individuals. Putman et al. (2007a) found acute cortisol administration in healthy participants to result in an increased performance bias for angry (compared to neutral) faces on a computerized object-relocation task, which was suggested to reflect a cortisol-induced increase in preferential processing of angry faces. In line with these results, in a study using a reaction time task to measure approach and avoidance responses to happy and angry faces, we found increased ERP amplitudes and relatively faster avoidance responses in reaction to angry faces after acute cortisol administration in high anxious

healthy participants (Van Peer et al., 2007). These results are in line with animal studies showing that high levels of cortisol are associated with increased fearful temperament and threat avoidance (Kalin et al., 1998a, b, 2000; Sapolsky, 1990), as well as with studies in humans showing increased threat processing (Mathews & Macleod, 1994) and threat avoidance (Roelofs et al., 2009a) in high anxious participants under stressful conditions.

The present study was set up as a follow-up of the study of Van Peer et al. (2007) in a group of participants with clinical (social) anxiety. This study is particularly relevant in the light of recent studies (Aerni et al., 2004; De Quervain & Margraf, 2008; Schelling et al., 2006; Soravia et al., 2006) showing effects of acute glucocorticoid administration with potential implications for the treatment of anxiety disorders such as PTSD and (spider and social) phobia. The results of one of these studies (Soravia et al., 2006) showed that cortisone administration 1 h before exposure to a socio-evaluative stressor resulted in a reduction in self-reported phobic fear during anticipation, exposure and recovery of this stressor in social phobic patients. Although the authors proposed inhibition of aversive memory retrieval as a likely mechanism underlying this fear-reduction, alternative processes such as an anxiolytic effect or modulation of other systems involved in the expression of fear may also play a role (see e.g., Putman et al., 2007b). Hence, it is important to asses the effects of cortisol administration on other key processes that have been implicated in the etiology and maintenance of anxiety disorders, such as attention towards threat stimuli and avoidance behavior (e.g., Bishop, 2008; Bögels & Mansell, 2004; Mathew & Ho, 2006; Mathews & MacLeod, 2005; Roelofs et al., 2009a).

Evidence for the presence of preferential processing of threatening information in high anxious subjects is primarily based on behavioral studies showing impairments in interference paradigms, such as Emotional Stroop or dot probe tasks (e.g., Bögels & Mansell, 2004; Mathews & MacLeod, 2005 for reviews). Another useful method to investigate this processing bias, however, is by recording event-related potentials (ERPs) from the scalp. Since ERPs are sensitive to both the extent (amplitude) and speed (latency) of cerebral processing, they can provide valuable information about early and rapid stages of attentional processing that is not reflected in behavioral measures (e.g., Bar-Haim et al., 2005; Thomas, Johnstone, & Gonsalvez, 2007). Hence they provide suitable tools to examine more closely the claim that threatening stimuli are associated

with enhanced attention in anxiety disorders, and to investigate the effects of cortisol administration on these processes.

ERP responses during processing of emotional material have been extensively studied using pictures of human faces, due to their social significance and affective salience (e.g., Bradley et al., 1997; Rolls, 2000). Results of these studies in healthy human subjects have shown very rapid effects (i.e., < 250 ms post-stimulus) suggesting early preferential processing of threat-related emotional faces (Ashley et al., 2004; Bar-Haim et al., 2005; Eger, Jedynak, Iwaki, & Skrandies, 2003; Eimer & Holmes, 2002; Williams et al., 2006), as well as modulation of later stages of ERP responses (Eimer & Holmes, 2002; Schupp et al., 2004; Williams et al., 2006).

Considering the suitability of the ERP-technique to study processing of emotional material, studies using ERPs to investigate threat processing in anxiety disorders are surprisingly scarce. Two recent studies investigated these processes using an emotional facial Stroop task in a clinical sample of patients with social anxiety disorder (Kolassa & Miltner, 2006; Kolassa, Kolassa, Musial, & Miltner, 2007). Abnormalities in processing of angry faces were found in one of these studies (Kolassa & Miltner, 2006), but not in the other (Kolassa et al., 2007). However, both studies focused only on occipito-temporal electrodes, and did not report on the early and late midline positive components described above, which are considered among the components most consistently demonstrating emotional expression ERP effects (see Holmes, Nielsen, & Green, 2008 for a review). Indeed, in a recent study Bar-Haim et al. (2005) found increased early positive (P2) amplitudes at the vertex for angry faces in high anxious compared to low anxious healthy participants, indicating enhanced early threat processing. Similarly, in our previous study we found the most pronounced effects of cortisol on threat processing in high anxious students on these early and late positive amplitudes (P150 and P300) at the vertex (Van Peer et al., 2007). For these reasons we focused on the P150 and P300 midline components in the present study.

In specific, we investigated the effect of acute cortisol administration on threat processing and behavioral avoidance in individuals with social anxiety disorder. Approach and avoidance reactions were assessed in reaction to positive and threatening social stimuli (i.e., happy and angry faces) using a reaction time affect-evaluation task (the approach-avoidance task, Rotteveel & Phaf, 2004), and threat processing was measured by recording event-related potentials during task performance. The

approach-avoidance task provides a reliable tool to investigate overt avoidance behavior (see e.g., Chen & Bargh, 1999; Rotteveel & Phaf, 2004; Solarz, 1960) and has been shown to be sensitive to social anxiety and cortisol manipulations in healthy populations (Heuer et al., 2007; Roelofs et al., 2005; Van Peer et al., 2007). Based on earlier findings with high anxious healthy participants (Van Peer et al., 2007) we expected relatively increased avoidance (i.e., slower approach or faster avoidance responses) and enhanced processing (i.e., increased early (P150) and later (P300) positive ERP amplitudes) of angry faces after cortisol administration.

Methods

Participants

Twenty-one unmedicated patients with SAD participated in the experiment for financial compensation (i.e., €40 and traveling expenses). Demographic variables and group characteristics are presented in Table 4.1. Patients were recruited at the outpatient anxiety departments of three community mental health centers and through advertisements on internet forums. Inclusion criteria were: a primary diagnosis of generalized SAD (according to DSM-IV criteria) and a total score > 60 at the Liebowitz Social Anxiety Scale (Liebowitz, 1987), right-handedness, normal or corrected-to-normal vision, and age 18-55 years. Exclusion criteria were current diagnosis of major depressive disorder, pregnancy or breast-feeding, clinical significant medical disease, past head injury with loss of consciousness > 5 min, use of psychotropic medication, use of corticosteroids in the 6 months prior to participation, use of cannabis more than once a week or use of any drugs other than cannabis in the 3 months prior to participation, and use of more than 3 glasses of alcohol or 20 cigarettes per day. Participants were instructed to minimize physical exercise, not to take large meals, chocolate or caffeine during the morning preceding the experiment, and not to eat, drink low pH drinks or smoke cigarettes in the hour before the start of the experiment, because these variables can affect saliva cortisol measures. All participants provided written informed consent prior to participation in the study, which was approved by the Medical Ethical Committee of the Leiden University Medical Center. Of the 21 patients tested, one had to be excluded because of missing reaction time data due to technical problems, leaving a total number of 20 participants (9 male, 11 female).

Table 4.1. Patient Characteristics (n =20).

Measure	*M*	*SD*
Age (years)	32.8	10.2
BMI	22.2	3.2
BDI	12.2	6.1
LSAS fear	42.1	8.0
LSAS avoidance	36.3	10.0
LSAS total	78.4	16.2
SPAI social phobia	131.0	21.0
SPAI agoraphobia	26.8	10.9
SPAI difference	104.2	21.6
STAI-trait	50.6	8.1
BIS	25.1	3.3
BAS total[a]	36.3	6.2
Axis-1 comorbidity[b]		
Comorbid anxiety disorder[c]	N = 1*	
Current mood disorder[d]	N = 0	
Past major depressive episode	N =7	

Note: (Scale range between parentheses). BMI = body mass index; BDI = Beck Depression Inventory (0-63); LSAS = Liebowitz Social Anxiety Scale (Fear 0-72, Avoidance 0-72, Total 0-144); SPAI = Social Phobia and Anxiety Inventory (Social Phobia 0-192, Agoraphobia 0-78); STAI = State-Trait Anxiety Inventory (20-80); BIS = Behavioral Inhibition Scale (7-28); BAS = Behavioral Activation Scale (13-52).

[a] NB n = 19 due to a missing value.

[b] Assessed using the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I).

[c] Including panic disorder, agoraphobia, specific phobia*, obsessive compulsive disorder, post-traumatic stress disorder and generalized anxiety disorder.

[d] Including current major depressive episode, mania, hypomania, dysthymic disorder, and bipolar disorder.

Participants were screened using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I: First et al., 1996) by a trained psychologist at the end of the first testing day to confirm diagnosis for social anxiety disorder and to exclude current major depressive disorder. Participants also completed Dutch versions of the Social Phobia and Anxiety Inventory (SPAI: Turner et al., 1989), the Beck Depression Inventory (Beck, Rush, Hollon, & Emery, 1979), the State-Trait Anxiety Inventory (Spielberger, 1983), and the Behavioral Inhibition and Behavioral Activation Scales (BIS/BAS: Carver & White, 1994). See Table 4.1 for questionnaire values.

Materials and procedure

For this study we used the same materials and procedure as reported by Van Peer et al. (2007). All participants were tested in a hydrocortisone (50 mg) and a placebo condition in a double-blind, within-subject crossover design. The order of cortisol or placebo administration was random and balanced over all participants. The two experimental sessions were 1 week apart. On the days of testing, participants

arrived at the laboratory at 12.15 p.m. After a short introduction, drugs were administered orally at 12.30 p.m., followed by a resting period of 1 h to allow for the cortisol to take effect. During this period, participants completed questionnaires and practiced with the response device for the approach-avoidance task, after which the electrodes for the electrophysiological measurements were placed. Subsequently, the experiment started with a short recording (~ 15 min) of the electroencephalogram (EEG) during rest, after which the approach-avoidance task was administered, followed by a number of additional cognitive tests of which the results will be reported elsewhere. During task performance, participants sat in an air-conditioned and sound-attenuated room in front of a computer monitor, and the experimenter sat in an adjacent room where the EEG apparatus was located.

Cortisol and subjective measures

Saliva samples were obtained using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Samples were obtained at four assessment points over a 165 min period, at respectively -5 min (T0), +60 min (T1), +120 min (T2), and +160 min (T3) with reference to capsule ingestion. Biochemical analysis of free cortisol in saliva was performed using a competitive electrochemiluminescence immunoassay (ECLIA, Elecsys 2010, Roche Diagnostics), as described elsewhere (Van Aken et al., 2003).

Self-reported mood (tension, fatigue, depression, anxiety, and activation at T0, T1, and T3) and motivation and concentration (directly before and after the AA-task) were rated on 100 mm visual analogue scales (VAS). In addition, state anxiety (STAI-state: Spielberger, 1983) was measured at T0 and T3.

Approach Avoidance task

In this affect-evaluation task (Rotteveel & Phaf, 2004; Van Peer et al., 2007), stimuli consisted of 60 grayscale photographs with happy and angry facial expressions (Ekman & Friesen, 1976; Matsumoto & Ekman, 1988; Lundqvist et al., 1998). Both the happy and the angry expression were taken from the same model (total of 30 models, 50% female). Each picture measured 12.4 cm × 8.9 cm ($h \times w$), and was presented against a black background at the center of a 15 in. computer screen at 70 cm viewing distance (see Figure 4.1).

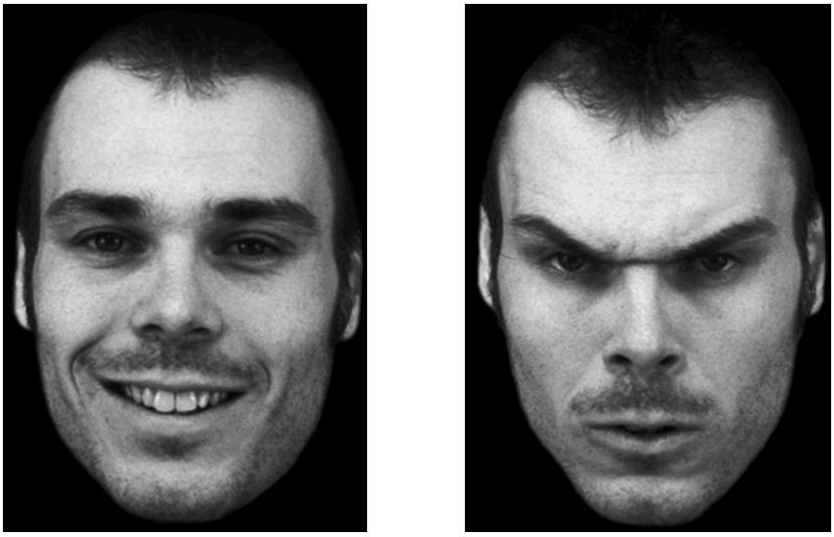

Figure 4.1. Examples of a happy and angry face stimulus used in the AA task.

Each trial started with the appearance of a central fixation point (100 ms), followed after an interval of 300 ms (black screen) by presentation of the stimulus (100 ms). The inter-stimulus-interval was randomized between 1500 and 2500 ms. Responses were registered by means of three buttons that were fixed to a vertical stand (see Rotteveel & Phaf, 2004, Figure 1) at the right side of the participant. Participants were instructed to push the "home" (middle) button loosely with the back of their right hand as long as no response was given, and to respond as fast and accurate as possible to the stimuli by releasing the home button and pressing one of the two response buttons (positioned 10.3 cm above and below the home button, allowing participants to simply flex or extend their arm in responding). After this, they had to return their hand to the home button.

Several studies (see e.g., Chen & Bargh, 1999; Rotteveel & Phaf, 2004; Solarz, 1960) have shown that arm flexion is associated with approach (as when pulling objects toward oneself), whereas arm extension is associated with avoidance (as when pushing something away). As a result, reaction times on the approach-avoidance task are typically faster when participants respond with arm flexion (approach) to positive stimuli and with arm extension (avoidance) to threatening stimuli (affect-congruent condition) than the other way around (affect-incongruent condition).

Participants received alternately an affect-congruent or an affect-incongruent instruction. The affect-congruent instruction indicated pressing the upper response button (arm flexion, approach movement) for happy faces and the lower button (arm

extension, avoidance movement) for angry faces. The affect-incongruent condition involved the opposite stimulus-response mapping (angry up, happy down). No reference was made in the instructions to congruence and incongruence, approach and avoidance, or arm flexion and extension.

The task consisted of four series of 60 trials, which were administered with either a congruent-incongruent-congruent-incongruent or an incongruent-congruent-incongruent-congruent order of instructions (counterbalanced across participants). Within each series all stimuli were presented once in a semi-randomized order (with a maximum succession of three happy or angry and three male or female pictures). Between each series participants performed an unrelated working memory task that served to ease the transition from affect-congruent to affect-incongruent instruction or vice versa. Each of the four series was divided into three blocks of 20 trials, with a short break (~30 s) between blocks, and was preceded by 20 practice trials of stimuli that were not included in the experimental series.

The task provided three behavioral measures: error rates (percentage incorrect responses) and two reaction time (RT) measures. The initiation time (IT) is the time between stimulus onset and the release of the home button. The movement time (MT) is the time between the release of the home button and the pushing of the response button.

Electrophysiological recording and data analyses

The electroencephalogram (EEG) was recorded from 19 scalp locations according to the international 10–20 system and referred on-line to C3/C4. An average earlobe reference was derived off-line. Vertical electro-oculogram (EOG) was recorded bipolarly from the supraorbital and the infraorbital ridge of the right eye, and horizontal EOG from the outer canthi of both eyes. The ground electrode was located at Fpz. EEG impedances were kept below 5 kΩ. The EEG and EOG signals were digitized at 500 Hz and segmented off-line (using Brain Vision Analyzer software,version 1.05, Brain Products GmbH, 1998–2004) into 1000 ms epochs, from 200 ms before to 800 ms after stimulus onset. Single trials were corrected for the effects of eye blinks and eye movements using a standard procedure (Gratton et al., 1983). Data were filtered digitally with a 0.01 Hz high-pass filter (24 dB/oct roll-off) and a 35 Hz low-pass filter (12 dB/oct). Artifact rejection was performed by removing epochs with activity below

0.50 μV and amplitudes exceeding ±75 μV in the C3, C4, Cz, F3, F4, Fz, P3, P4, and Pz electrode channels (average 1.2% of total dataset).

Separate averages were computed for happy and angry faces as a function of arm movement (approach/flex or avoid/extend). Based on the results of our previous study (Van Peer et al., 2007) analyses focused on the P150 and P300 components at midline electrodes (Fz, Cz, Pz). Peak amplitudes of these components were identified as local maximum relative to baseline in two successive time windows: P150 (the first major positive wave occurring 120–200 ms post-stimulus) and P300 (second major positive wave, 270– 400 ms). Time windows for peak detection were based on visual inspection of the grand average ERPs, averaged across all participants and categories. Incorrect responses and responses with ITs < 150 ms or > 1000 ms (total 4.7% of trials) were excluded from the RT and ERP analyses. Error rates were consistently low in all conditions (M = 3.0 ±1.8 %) and are therefore not reported.

The influence of cortisol administration on subjective measures, salivary cortisol, AA-task performance, and ERP peak amplitudes were tested with repeated measures analyses of variance (ANOVAs rm) using the Statistical Package for the Social Sciences (SPSS 14.0, SPSS Inc., 1989–2005). Since previous studies have shown that both ERPs related to processing of threatening faces (see e.g., Bar-Haim et al., 2005; Holmes et al., 2008) and the effects of cortisol on approach-avoidance tendencies (Van Peer et al, 2007; Roelofs et al., 2005) can be moderated by individual differences in anxiety, we included social anxiety (SPAI social phobia score) as a continuous variable (ANCOVA) in our behavioral and ERP analyses (see Judd et al., 2001). Significant effects including this variable were further investigated by calculating Pearson correlations between this factor and the within-subject effects. All statistical analyses employed a two-tailed alpha of .05. Effect sizes are reported as proportion of explained variance (partial eta squared [η^2]). The Greenhouse-Geisser correction was used when appropriate (epsilon [ε]).

Results

Cortisol and subjective measures

Salivary cortisol (nmol/L) measures (see Table 4.2) were skewed and therefore log transformed before statistical analysis. The results of a 2 × 4 ANOVA rm with

Table 4.2. Mean free salivary cortisol levels (nmol/L) after placebo and cortisol administration relative to time of capsule intake (t = 0)

Time (min)	Placebo		Cortisol	
	M	*SD*	*M*	*SD*
-5	9.7	3.5	9.7	3.6
+60***	8.3	2.7	294.2	213.5
+120***	7.3	2.5	230.0	186.5
+165***	6.7	2.4	151.2	155.1

Note: N= 17 due to missing values (unreliable saliva measurements n=2) and missing data AA-task (*N*=1). *** placebo vs. cortisol p<.001

Condition (placebo, cortisol) and Time (T0, T1, T2, T3) yielded a significant interaction of Condition × Time ($F(3,48) = 78.47$, $p = .000$, $\eta^2 = 0.83$). This result indicates that, as expected, salivary cortisol levels did not differ between conditions before capsule intake (T0: $F(1,17) = .01$, $p = .92$), but were significantly increased after cortisol administration compared to placebo from one hour after capsule intake until the end of the experiment (T1: $F(1,16) = 147.85$, $p = .000$; T2: $F(1,17) = 214.16$, $p = .000$; T3: $F(1,17) = 124.08$, $p = .000$). Note that the AA-task was administered between T1 and T2 (i.e., between one and two hours after capsule intake).

To investigate effects of cortisol administration on subjective mood (data not shown) we conducted separate ANOVAs rm with Condition (placebo, cortisol) × Time for STAI-state (T0, T3) and VAS tension, fatigue, depression, anxiety, and activation (T0, T1, T3). Results showed no significant main or interaction effects of Condition on STAI-state anxiety, VAS tension, fatigue, depression, or anxiety (all $F< 2.4$, $p > .14$). We did find a significant main effect of Condition on VAS activation ($F(1,18) = 7.65$, $p = .013$). However, follow up analyses revealed that reported activation was higher in the placebo than the cortisol condition before capsule intake (T0: $F(1,18) = 9.59$, $p = .006$). Consequently, to control for pre-drug differences in activation level we performed an additional analysis using the pre-drug activation level (average of T0 in placebo and cortisol condition) as a covariate. The results of this analysis revealed no difference between conditions in post-drug activation levels ($F(1,17) = 0.26$, $p = .62$), indicating that cortisol administration did not affect mood.

Behavioral results

Initiation times (IT)

For the initiation times we found the expected congruency effect: A significant Emotion × Arm movement interaction ($F(1,18) = 5.64$, $p = .029$, $\eta^2 = .24$) showed that patients were faster in initiating affect-congruent (approach happy: M = 495 ± 62 ms; avoid angry: M = 509 ± 59 ms) than affect-incongruent arm movements (avoid happy: M = 524 ± 68 ms ; approach angry: M = 521 ± 64 ms). In contrast to our expectations, this AA congruency effect was not modulated by cortisol administration (Condition × Emotion × Arm movement: $F(1,18) = 1.05$, $p = .31$). For Social anxiety we found a significant main effect, reflecting faster initiation times for patients with higher levels of social anxiety ($F(1,18) = 6.59$, $p = .019$, $\eta^2 = .27$, $R = -0.52$), but no modulation of the AA congruency effect (Emotion × Arm movement × Social anxiety: $F(1,18) = 2.48$, $p = .13$). The results showed no other significant effects on initiation times (All $F < 1.83$, $p > .19$).

Movement times (MT)

There was a main effect of Emotion ($F(1,18) = 4.80$, $p = .042$), indicating faster movement times for happy than angry faces. In line with the results on initiation times, this effect was modulated by a trend towards an interaction of Emotion × Arm movement ($F(1,18) = 3.62$, $p = .073$, $\eta^2 = .17$), suggesting that patients tended to be faster in executing affect-congruent (approach happy: M = 189 ± 89 ms; avoid angry: M = 184 ± 74 ms) than affect-incongruent arm movements (avoid happy: M = 188 ± 68 ms; approach angry: M = 199 ± 77 ms). In addition, we found a significant interaction of Emotion × Social anxiety ($F(1,18) = 5.60$, $p = .029$), as well as an interaction of Emotion × Arm movement × Social anxiety ($F(1,18) = 5.39$, $p = .032$, $\eta^2 = 0.23$). Follow up analyses to determine the nature of this interaction revealed a significant interaction of Emotion × Social anxiety for approaching ($F(1,18) = 10.70$, $p = .004$, $\eta^2 = .37$) but not for avoiding arm movements ($F(1,18) = .048$, $p = .83$). Calculation of the Pearson correlation between the social anxiety levels and the emotion difference score (MT angry minus MT happy) for approach movements showed that the direction of this relation was positive (R = 0.61; for avoidance R = 0.05), indicating that high levels of social anxiety were associated with significantly longer movement times for approach of angry compared to happy

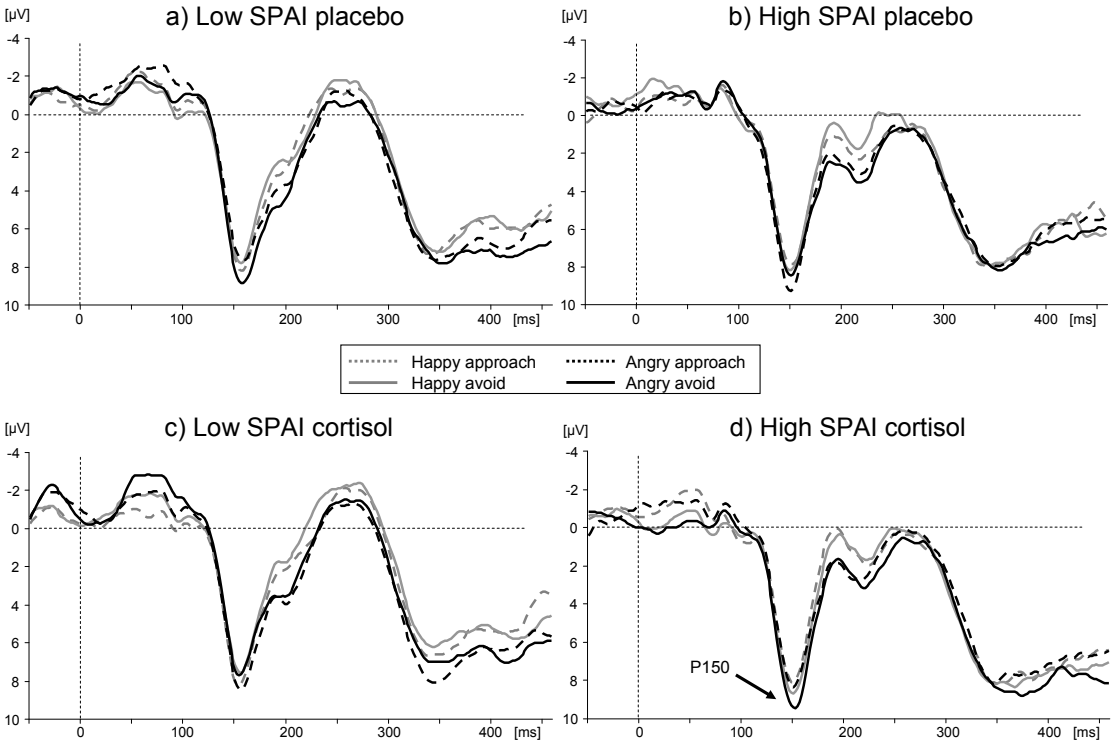

Figure 4.2. Stimulus synchronized grand average ERP waveforms at Pz electrode. To show the effects of cortisol and social anxiety, waveforms are presented separately for the placebo (upper panel) and cortisol (lower panel) conditions, and for patients with relatively low (left) and patients with relatively high (right) social anxiety scores. For presentation purposes, patient groups are formed based on a median split on SPAI social phobia scores (Median = 130.3, N = 10 in each group). Note that in the statistical analyses social anxiety was included as a continuous measure and not as a group factor. Results showed a significant Condition by Arm movement by Social anxiety interaction, reflecting relatively increased P150 amplitudes for avoidance compared to approach after cortisol administration in patients with higher levels of social anxiety. This effect was most strong (and only significant) for angry faces.

faces. Follow-up analyses of the Movement × Social anxiety interaction separately by emotion were not significant (Happy: $F(1,18) = 3.24$, $p = .089$; Angry: $F(1,18) = 0.85$, $p = .37$). We did not find any significant effect of cortisol administration on movement times (all $F<0.91$, $p > 0.34$).

ERP results

Figure 4.2 presents grand average ERPs at Pz electrode (where the effects were most pronounced) on trials with approach and avoidance responses in reaction to happy and angry faces.

P150 amplitude

For the P150 on midline electrodes we found a significant interaction of Condition × Arm movement ($F(1,18) = 6.97$, $p = .017$), which was further modulated by a significant

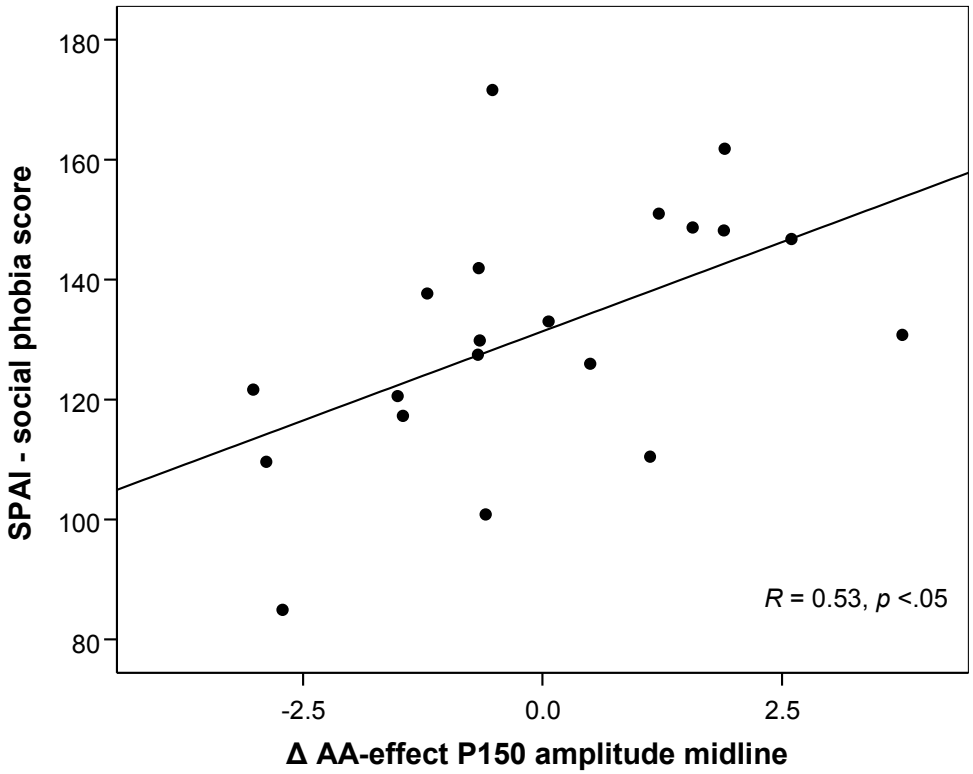

Figure 4.3. Correlation between social anxiety (SPAI- social phobia scale) and cortisol-induced change in P150 amplitude for avoidance compared to approach (i.e. [Δ Avoid-Approach cortisol] minus [Δ Avoid-Approach placebo]). Positive numbers for the ΔAA-effect indicate larger P150 amplitudes on midline electrodes (Fz, Cz, Pz) for avoidance than approach in the cortisol condition, compared to the placebo condition. The scatterplot shows that high levels of social anxiety are associated with increased P150 ΔAA-effects after cortisol administration, indicating an increase in processing of emotional faces during avoidance compared to approach.

3-way interaction of Condition × Arm movement × Social anxiety[1] ($F(1,18) = 6.99$, $p = .016$, $\eta^2 = 0.28$). Follow-up analyses showed that this finding reflects a significant Arm movement × Social anxiety interaction after cortisol administration ($F(1,18) = 6.39$, $p = .021$, $\eta^2 = 0.26$) but not after placebo ($F(1,18) = 1.52$, $p = .23$). In addition, the interaction of Condition × Social anxiety was marginally significant for avoidance movements ($F(1,18) = 3.99$, $p = .061$), but not significant for approach movements ($F(1,18) = 0.36$, $p = .56$). The significant 3-way interaction indicates that the effect of cortisol administration on P150 amplitude during avoidance compared to approach correlated significantly with the patients' social anxiety levels. Pearson correlations between social

[1] A second analysis for P150 and P300 amplitude was conducted with F3, F4, C3, C4, P3, and P4 as additional electrodes, and with laterality (left, midline, right) as an additional factor. This analysis only showed one significant interaction with laterality for P150 amplitude, i.e. Condition x Arm movement x Social anxiety x Laterality ($F(2,36) = 3.42$, $p=.046$, $\varepsilon = 0.96$, $\eta^2 = 0.16$). Post hoc testing revealed that the interaction of Condition x Arm movement x Social anxiety was most pronounced at the midline electrodes, and only marginally significant at the left ($F(1,18) = 3.06$, $p=.097$) and right lateral electrodes ($F(1,18) = 3.23$, $p=.089$). Therefore, only results of midline electrodes are presented.

anxiety levels and the cortisol-induced change in P150 amplitude for avoidance compared to approach (i.e., [P150 amplitude avoid - approach cortisol] minus [P150 amplitude avoid - approach placebo]) showed that the direction of this correlation was positive (R = 0.53), indicating that patients with higher social anxiety levels showed a larger cortisol-induced increase in P150 amplitude for avoidance compared to approach (see Figure 4.3). We found no significant interaction with Emotion (all $F < 2.3$, $p > .14$), suggesting that this effect was not significantly different for happy and angry faces. However, since we specifically expected effects for processing of threat, we conducted a planned comparison to investigate whether this effect would hold when testing angry faces separately. Indeed, the effect was most strong and significant for the angry faces (R = 0.52, p = .019) whereas it was not significant for happy faces (R = 0.19, p = .42).

We found no significant effects on P300 midline amplitudes (all $F < 2.14$, $p > .16$).

Discussion

The aim of the present study was to investigate the effects of cortisol administration on threat processing and approach and avoidance behavior in a clinical sample of patients with generalized social anxiety disorder. In line with earlier findings of a very similar study with high anxious healthy participants (Van Peer et al., 2007) we expected relatively increased avoidance (i.e., slower approach or faster avoidance responses) and enhanced processing (i.e., increased early (P150) and later (P300) positive ERP amplitudes) of angry faces after cortisol administration.

First, our behavioral results showed the expected emotion by arm movement interaction (congruency effect) in reaction times, indicating that the patients were faster in initiating affect-congruent (approach happy, avoid angry) than affect-incongruent (approach angry, avoid happy) arm movements, consistent with the findings of several previous studies in healthy participants (Roelofs et al., 2005; Rotteveel & Phaf, 2004; Van Peer et al., 2007). In contrast to our expectations and previous findings (Van Peer et al., 2007), however, we did not find an effect of cortisol administration on initiation times in the present study. Since the experimental procedure was the same as in our previous study it is difficult to account for the current absence of this effect, although it could be due to the heterogeneity of the current sample compared to the relatively homogeneous male sample of our previous study.

For the movement times the emotion by arm movement interaction was modulated by the severity of patients' social anxiety. Post hoc testing revealed that the execution of approach movements to angry faces (compared to happy faces) was significantly slower for patients with higher levels of social anxiety. Reaction times for affect-incongruent responses, such as approaching an angry face, reflect the costs of inhibiting an intuitive response tendency, i.e., to avoid the angry face, in favor of the instructed response (Roelofs et al.,2009b). This slowing of approach responses towards angry faces is therefore consistent with a relatively increased tendency to avoid threat in patients with higher levels of social anxiety.

Most importantly, results of the ERP analyses showed a significant interaction of cortisol by social anxiety on early positive (P150) amplitudes: Cortisol administration resulted in a significant increase in P150 amplitudes during avoidance compared to approach of emotional faces for patients with high levels of social anxiety. This result is largely in line with our previous finding of enhanced P150 amplitudes during avoidance of angry faces after cortisol administration in high trait avoidant healthy participants (Van Peer et al., 2007). Although the lack of a significant interaction with stimulus emotion in the present study implies that this effect was not significantly different for happy and angry faces, the effect was still significant when tested for the angry (but not the happy) faces separately. This is in line with the findings of our previous study, as well as with other studies showing sensitivity to social threat in patients with social anxiety (see e.g., Bishop, 2008; Bögels & Mansell, 2004; Mathew & Ho, 2006; Mathews & MacLeod, 2005).

Increased amplitudes of early (as well as late) positive midline ERP components in reaction to threat-related emotional faces have been consistently reported in studies in healthy participants, and are generally interpreted as reflecting increased allocation of processing resources to motivationally significant stimuli (Bar-Haim et al., 2005; Eimer & Holmes, 2002; Eimer et al., 2003; Williams et al., 2006). Furthermore, Bar-Haim et al. (2005) found enhanced P2 amplitudes to angry faces in high compared to low anxious healthy participants, indicating that early threat processing is modulated by trait anxiety level (although cf. Holmes et al., 2008; Moser, Huppert, Duval, & Simons, 2008). The results of our studies suggest that this process is sensitive to cortisol administration, resulting in enhanced processing of social threat in high socially anxious participants. This is in line with behavioral findings indicating increased preferential

processing of angry faces after cortisol administration (Putman et al., 2007a). Most importantly, the present study is the first to show an effect of cortisol administration on threat processing in a clinical sample of patients with generalized social anxiety disorder.

Although the timing of the ERP effect in the present study suggests that early stages of information processing are involved, it most likely does not reflect pre-attentive classification processes (Eimer & Holmes, 2007). Instead, such an early midline positive ERP effect is proposed to reflect higher order and attention-dependent processing in neocortical areas, where representations of emotional content are generated in a strategic and task-dependent fashion for the adaptive intentional control of behavior (Eimer & Holmes, 2007). Recent findings by Amodio & Potanina (2008) support this notion by showing that the P200 component reflects motivated attention to cues related to response control. Interestingly in this respect is our finding, in the present as well as our previous study (Van Peer et al., 2007), that after cortisol administration in high anxious participants the P150 amplitude in reaction to angry faces was significantly higher for avoidance compared to approach movements. This implies that the effect of cortisol administration on early threat processing is also modulated by the behavioral response mode, suggesting that early processing is indeed related to behavioral control mechanisms. In our design, affect-congruent and affect-incongruent responses were blocked in separate instruction conditions, which may have resulted in priming of response-congruent stimulus processing.

Together our findings suggest that cortisol-induced enhancement of emotional face processing depends on symptom severity and motivational context in SAD. There are, however, some limitations that should be discussed. First, the present study aimed to replicate and extend previous findings in high anxious healthy participants (Van Peer et al., 2007) to patients with clinical SAD, and therefore we did not include an additional non-anxious control group. The current within-subject design allowed us to control for individual differences in symptom severity, which proved to be an important moderating factor. Nevertheless, a matched control group would have offered more information regarding the specificity of the effects of cortisol on threat processing for social anxiety. We cannot draw conclusions about this specificity based on the present study. The results of previous studies of our group, investigating the effects of cortisol on approach-avoidance in high versus low anxious healthy participants (Van Peer et al.,

2007) and in patients with SAD versus patients with PTSD and healthy controls (Roelofs et al., 2009a), however do suggest that the association of high cortisol levels with increased social avoidance tendencies may be specific to high socially anxious individuals.

Second, in contrast to our expectations and previous findings (Van Peer et al., 2007), we did not find an effect of cortisol on the behavioral results in the present study. As we suggested above, this could be due to the heterogeneity of the current sample compared to the relatively homogeneous male sample of our previous study. On the other hand, modulation of ERP components in absence of behavioral effects has been reported by several other authors (see e.g., Bar-Haim et al., 2005; Holmes et al., 2008; Thomas et al., 2007) and it has been suggested that ERPs may provide a more sensitive measure of attentional biases compared to reaction times. In addition, early positive ERP components in particular have been associated with enhanced attentional vigilance for threat-related material in high anxious participants (e.g., Bar-Haim et al., 2005; Holmes et al., 2008). In both of our studies, the effects of cortisol administration on ERP components related to threat processing were more pronounced on these early (P150) compared to later (P300) amplitudes.

Together, our findings suggest that a mechanism of early threat processing -that is enhanced by cortisol in high anxious healthy participants- is similarly affected in patients diagnosed with generalized SAD. Although the present study is the first ERP study on cortisol administration in SAD and the results should be replicated to allow definite conclusions, these findings may have some valuable methodological and clinical implications.

First, it underscores the usefulness of the ERP methodology as a sensitive measure for both the study of attentional processes in anxiety (e.g., Bar-Haim et al., 2005; Thomas et al., 2007) and the study of the effects of cortisol on motivational processes.

Second, our findings suggest that it is important to take motivational processes into consideration when investigating effects of cortisol and anxiety on threat processing. A few recent studies have investigated ERPs related to threat processing in anxiety (Bar-Haim et al., 2005; Holmes et al., 2008; Kolassa & Miltner, 2006; Kolassa et al., 2007; Moser et al., 2008), and results thus far have been rather inconsistent (see Holmes et al., 2008 for a review). Our finding that after cortisol administration the P150

amplitude for angry faces was significantly higher for avoidance compared to approach movements suggests that it is important to take motivational behavior into account (see also Amodio & Potanina, 2008). This may help resolve inconsistencies not only in the ERP literature on emotional processing and anxiety, but also in the reported effects of cortisol administration on ERPs related to stimulus processing (Born, Hitzler, Pietrowsky, Pauschinger, & Fehm, 1988; Born, Kern, Fehm-Wolfsdorf, & Fehm, 1987; Hartmann et al., 1995; Hsu, Garside, Massey, & McAllister-Williams, 2003; Kopell, Wittner, Lunde, Warrick, & Edwards, 1970).

Third, the interaction with social anxiety in the present study indicates that it may be important to take individual differences in symptom severity into account when studying emotional processing in patient groups (see also Kolassa & Miltner, 2006; Roelofs et al. 2009a) as has been demonstrated earlier in samples of healthy participants (see Holmes et al., 2008; Roelofs et al., 2005; Van Peer et al., 2007).

Fourth, our finding that cortisol administration affects threat processing in a clinical sample of patients diagnosed with generalized SAD is important in light of the recent interest in cortisol administration as a possible treatment for anxiety disorders (see De Quervain & Margraf, 2008; Soravia et al., 2006). Our results indicate that in addition to memory processes or subjective fear responses, as put forward earlier by several authors (e.g., De Quervain & Margraf, 2008; Soravia et al., 2006), cortisol administration can also affect initial attention-related threat processing in social anxiety, which has been proposed as another important mechanism in the etiology and maintenance of this disorder (see e.g., Bögels & Mansell, 2004; Mathews & MacLeod, 2005 for reviews). At first sight our findings may seem in contrast with the findings of Soravia et al. (2006) which indicated a reduction in subjective fear in SAD after cortisol administration. There are, however, some important differences between the studies:

First, they focus on different aspects (phases) of emotional processing. Whereas Soravia et al. (2006) studied subjective fear responses (proposed to be mediated by retrieval of fear memory), the main effect of cortisol in our study was found on early attention processes (i.e., P150). It is presently unclear how enhanced early processing of threat cues, as reflected by ERPs, is exactly related to subsequent subjective fear responses. We suggest that enhanced early ERP amplitudes do not themselves indicate increased anxiety, but rather reflect increased vigilance or motivated attention to the threat stimuli (a view supported by studies where such increased attention to threat

occurred in the context of anger-related approach motivation -see e.g., Bertsch et al., 2008; Putman et al., 2007a). According to cognitive theories of anxiety, not only the initial vigilance but also subsequent higher order processes (e.g., coping behavior) are relevant for predicting emotional reactions to the threat stimuli (see e.g., Mathews & Mackintosh, 1998). For instance, if increased threat processing is followed by increased avoidance behavior, this can reduce immediate subjective anxiety (see the vigilance-avoidance hypothesis, e.g., Mogg et al., 1997), although in the long term this behavior may maintain anxiety by preventing reappraisal of the threat.

Another important difference between our studies is the context in which the effects of cortisol were studied. Whereas Soravia et al. (2006) studied the effects of cortisol administration during exposure to a stressful situation, in our study the testing situation was relatively relaxed. As shown by Tops, Van Peer, Wester, Wijers, & Korf (2006), the effects of cortisol administration are context-sensitive and can have opposite effects depending upon the stressfulness of the testing situation. Although the literature on this topic is scarce, there are several studies suggesting that cortisol administration in a stress context can lead to a reduction in negative mood and avoidance motivation (see e.g., Het & Wolf, 2007; Reuter, 2002; Soravia et al., 2006; Tops et al., 2006), whereas cortisol administration in absence of stress results in enhanced processing of threatening information and relatively increased avoidance motivation (see Putman et al. 2007a; Tops et al., 2005, 2006; Van Peer et al., 2007; cf. Buchanan, Brechtel, Sollers, & Lovallo, 2001). This notion that effects of cortisol administration are context-dependent is in line with extensive animal literature (see e.g., De Kloet et al., 1999; Lupien et al., 2007). More research is warranted to investigate the effects of cortisol administration on different phases of emotional processing and the effect of different experimental contexts.

To conclude, this study is the first to investigate the effect of cortisol administration on threat processing and avoidance in a clinical sample of patients with generalized social anxiety disorder, and shows that cortisol-induced enhancement of emotional face processing in these patients depends on symptom severity and motivational context.

Chapter 5

Psychophysiological evidence for cortisol-induced reduction in early bias for implicit social threat in social phobia

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J.M. van Peer, Ph. Spinhoven & K. Roelofs

Abstract

The stress hormone cortisol is important for the regulation of social motivational processes. High cortisol levels have been associated with social fear and avoidance, which play an important role in social anxiety disorder (SAD), as does hypervigilant processing of social threat. However, causal effects of cortisol on threat processing in SAD remain unclear. In an event-related potential (ERP) study we investigated the effects of cortisol on task-irrelevant (implicit) processing of social threat in SAD, exploring the temporal dynamics as well as the role of symptom severity and stimulus awareness. Angry face processing was measured in participants with clinical SAD after double-blind, within-subject oral administration of cortisol (50 mg) and placebo, using a masked and an unmasked emotional Stroop task. Both tasks showed significantly increased P2 midline ERP amplitudes for angry compared to neutral and happy faces in the placebo condition, reflecting an early attentional bias for social threat in SAD. Furthermore, cortisol administration significantly decreased P2 amplitudes for masked angry faces. This effect correlated with social anxiety, showing stronger decreases in patients with higher levels of social anxiety. These results indicate a highly specific effect of cortisol on early motivated attention to social threat and, together with previous findings, highlight the importance of motivational context (stimulus- or task-relevance) as well as symptom severity.

Introduction

Social anxiety disorder (SAD, or social phobia) is characterized by intense fear and avoidance of social situations. Cognitive models of SAD suggest that it is associated with biases in attending to threat-related information, and that these information-processing biases may be implicated in the development and maintenance of anxiety symptoms (see e.g., Clark & Wells, 1995; Mobini & Grant, 2007; Rapee & Heimberg, 1997; Schultz & Heimberg, 2008). There is, however, no consensus about the specific processing stages in which these biases occur. In the present study we aim to gain more insight in the temporal dynamics of biased processing of social threat in SAD, by investigating event-related potentials (ERPs) during masked and unmasked versions of a pictorial emotional Stroop task. In addition, recent neurobiological accounts propose that increased social fear and avoidance as found in SAD may be related to high levels of the stress hormone cortisol (e.g., Condren et al., 2002; Hermans & Van Honk, 2006; Roelofs et al., 2009a). However, causal effects of cortisol on threat processing remain unclear. Therefore, our second purpose was to investigate the effect of cortisol administration on social threat processing in SAD.

Although a wide range of studies has provided empirical evidence for biased processing of social threat in SAD (see e.g., Bar-Haim et al., 2007; Mobini & Grant, 2007 for reviews), results are conflicting with regard to the direction of this bias. Several studies have found a bias *towards* threat (or threat vigilance) in socially anxious participants, as indicated by longer reaction times (RT) for color-naming threat vs. neutral stimuli in emotional Stroop tasks (e.g., Amir, Freshman, & Foa, 2002; Mattia, Heimberg, & Hope, 1993; Spector, Pecknold, & Libman, 2003) or speeded responses to threat cues in spatial attention paradigms such as dot-probe or visual search tasks (e.g., Asmundson & Stein, 1994; Gilboa-Schechtman, Foa, & Amir, 1999; Mogg & Bradley, 2002; Mogg et al., 2004; Musa, Lepine, Clark, Mansell, & Ehlers, 2003). Other studies, however, reported a bias *away* from threat (or threat avoidance) in socially anxious participants, as indicated by shorter RTs for threat vs. neutral stimuli in emotional Stroop tasks (Putman, Hermans, & Van Honk, 2004) or longer RTs to threat cues in dot-probe tasks (e.g., Chen, Ehlers, Clark, & Mansell, 2002; Mansell et al., 1999). Although these discrepant findings may be

in part related to variation in experimental paradigms (e.g., Stroop vs. dot-probe) or stimulus materials (words vs. face pictures) (see Bar-Haim et al., 2007; Mobini & Grant, 2007), an alternative explanation is that the direction of the attentional bias is related to the timing of the effects that are tapped by a specific paradigm, and depends on different underlying cognitive processes. According to the hypervigilance-avoidance hypothesis (Mogg et al., 1997; Mogg & Bradley 2002), anxious participants may initially orient towards threat, but subsequently direct their attention away in order to reduce their anxiety levels. Although some recent studies provided support for this hypothesis in individuals with SAD (Amir et al., 1998; Mogg et al., 2004), RT data as used in these studies reflect the product of a range of cognitive processes and may therefore be less sensitive for differentiating between biases in early or late stages of information processing. In contrast, event-related potentials (ERPs) recorded from the scalp provide a continuous and high temporal resolution measure of the extent (amplitude) and speed (latency) of cerebral processing, and are therefore particularly suitable for a more refined investigation of the time course of attention allocation to stimuli during emotional processing.

ERPs have been widely used to study processing of emotional material, often including pictures of angry or fearful faces as social threat stimuli. Results of these studies in healthy participants have shown very rapid effects (i.e., < 250 ms post-stimulus) suggesting early preferential processing of threat-related emotional faces (Ashley et al., 2004; Bar-Haim et al., 2005; Eger et al., 2003; Eimer & Holmes, 2002; Williams et al., 2006), as well as modulation of later stages of threat processing (Eimer & Holmes, 2002; Schupp et al., 2004; Williams et al., 2006). However, studies using ERPs to investigate threat processing in SAD are relatively scarce. In three recent studies Kolassa et al. (Kolassa & Miltner, 2006; Kolassa et al., 2007, 2009) investigated threat processing in patients with SAD using angry compared to neutral and happy faces. Two of these studies (Kolassa et al., 2007, 2009) showed no differential processing of angry faces in SAD during either color or explicit emotion identification of schematic faces. Results of the other study (Kolassa & Miltner, 2006) showed biased early processing of angry photographic faces in patients with SAD, as reflected by enhanced right temporo-parietal N170 amplitudes, during explicit emotion identification but not when emotion processing was implicit (i.e., during gender identification). As suggested by Bar-Haim et al. (2007), biased processing during explicit emotion identification may be contingent on

the stimulus being task-relevant, which hinders the generalizability of such findings. Furthermore, both of these studies (Kolassa & Miltner, 2006; Kolassa et al., 2007) focused only on occipito-temporal and parietal electrodes and did not report on the early and late midline positive ERP components that have consistently demonstrated emotional expression effects in healthy participants (see Holmes et al., 2008), and were also shown to be enhanced during implicit angry face processing in high anxious healthy participants (Bar-Haim et al., 2005). Thus, the first aim of the present study was to gain more insight in the temporal dynamics of implicit threat processing in SAD by investigating midline positive ERPs during color-naming in a modified emotional Stroop task with photographic faces. We also included a masked version of this task, to investigate threat processing biases under conditions of restricted awareness.

Our second aim was to investigate the effects of cortisol administration on implicit threat processing in SAD. The stress hormone cortisol plays an important role in the regulation of social motivational processes (e.g., Kalin et al., 1998a; Roelofs et al., 2005, 2009a; Sapolsky et al., 2000; Van Honk et al., 1998, 2000; Van Peer et al., 2007), and high cortisol stress-responses have been associated with increased threat avoidance in SAD (Roelofs et al., 2009a). Furthermore, cortisol administration has recently been proposed as a possible treatment for SAD, because it reduced self-reported anxiety in social phobic patients during exposure to socio-evaluative threat (e.g., De Quervain & Margraf, 2008; Soravia et al., 2006). However, relatively little is known about the effects of cortisol administration on cognitive-emotional processes such as attention to threat. A few recent studies, using explicit emotion evaluation paradigms, showed that cortisol administration can increase angry face processing, especially in high anxious individuals (Putman et al., 2007a; Van Peer et al., 2007; Van Peer, Spinhoven, Van Dijk, & Roelofs, 2009). In the present study, we investigate whether cortisol administration has similar effects on the *implicit* (task-irrelevant) processing of threat.

Implicit threat processing will be measured by recording ERPs during color-identification of angry, happy and neutral faces in both a subliminal (masked) and a supraliminal (unmasked) version of a pictorial emotional Stroop task. The tasks are administered in both a placebo and a cortisol administration condition, using a within-subject design. Based on the hypervigilance-avoidance hypothesis (e.g., Mogg et al., 1997) and previous findings of enhanced early threat processing in high anxious participants (e.g., Bar-Haim et al., 2005; Holmes et al., 2008) we expect to find relatively

increased early positive midline amplitudes for angry faces in the placebo condition. This effect may be followed by shorter color-naming latencies for angry faces, reflecting threat avoidance (see Putman et al., 2004). Furthermore, we will test whether, and at which stage, this threat processing is affected by cortisol administration.

Methods

Participants

Eighteen unmedicated patients with SAD participated in the experiment for financial compensation. Group characteristics are presented in Table 5.1. Patients were recruited at the outpatient departments of three community mental health centers and through advertisements on internet forums. Inclusion criteria were: a primary diagnosis of generalized SAD (according to DSM-IV criteria) and a total score > 60 at the Liebowitz Social Anxiety Scale (Liebowitz, 1987), right-handedness, normal or corrected-to-normal vision, and age 18-55 years. Exclusion criteria were current diagnosis of major depressive disorder, pregnancy or breast-feeding, clinical significant medical disease, past head injury with loss of consciousness > 5 min, use of psychotropic medication, use of corticosteroids in the six months prior to participation, use of cannabis more than once a week or use of any drugs other than cannabis in the three months prior to participation, and use of more than three glasses of alcohol or 20 cigarettes per day. Participants were instructed to minimize physical exercise, not to take large meals, chocolate or caffeine during the morning preceding the experiment, and not to eat, drink low pH drinks or smoke cigarettes in the hour before the start of the experiment, because these variables can affect saliva cortisol measures. All participants provided written informed consent prior to participation in the study, which was approved by the Medical Ethical Committee of the Leiden University Medical Center. Of the 18 patients tested, one had to be excluded because of missing reaction time data due to technical problems, leaving a total number of 17 participants (7 male, 10 female).

Participants were screened using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I: First et al., 1996) by a trained psychologist at the end of the first testing day to confirm diagnosis for social anxiety disorder and to exclude current major depressive disorder. Participants also completed Dutch versions of the Social

Table 5.1. Patient Characteristics (n =17)

Measure	*M*	*SD*
Age (years)	31.4	10.0
BDI	12.7	6.1
LSAS fear	43.2	7.1
LSAS avoidance	36.5	10.8
LSAS total	79.7	15.9
SPAI social phobia	132.4	20.9
SPAI agoraphobia	26.4	10.0
SPAI total	106.0	21.3
Axis-1 comorbidity[a]		
Comorbid anxiety disorder[b]	*n* = 0	
Current mood disorder[c]	*n* = 0	

Note: (Scale range between parentheses). BDI = Beck Depression Inventory (0-63); LSAS = Liebowitz Social Anxiety Scale (Fear 0-72, Avoidance 0-72, Total 0-144); SPAI = Social Phobia and Anxiety Inventory (Social Phobia 0-192, Agoraphobia 0-78, Total = SP - Ag).

[a] Assessed using the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I).

[b] Including panic disorder, agoraphobia, specific phobia, obsessive compulsive disorder, post-traumatic stress disorder and generalized anxiety disorder.

[c] Including current major depressive episode, mania, hypomania, dysthymic disorder, and bipolar disorder.

Phobia and Anxiety Inventory (SPAI: Turner et al., 1989), and the Beck Depression Inventory (Beck et al., 1979). See Table 5.1 for questionnaire values.

Materials and procedure

All participants were tested in a cortisol and a placebo condition in a double-blind, within-subject crossover design. The order of cortisol (50 mg hydrocortisone) or placebo (primogel FNA) administration was random and balanced over all participants. The two experimental sessions were one week apart. On the days of testing, participants arrived at the laboratory at 1215h. After a short introduction, drugs were administered orally at 1230h, followed by a resting period of 1h to allow for the cortisol to take effect. During this period, participants completed questionnaires, after which the electrodes for the electrophysiological measurements were placed. The experiment started with a short recording of the resting state electroencephalogram (EEG), followed by a number of additional cognitive tests of which the results will be reported elsewhere (see e.g., Van Peer et al., 2009). The emotional Stroop task (~ 15 min) was administered at the end of the experiment, approximately 2.5h after capsule intake. During task performance, participants sat in an air-conditioned and sound-attenuated room in front of a computer

monitor, and the experimenter sat in an adjacent room where the EEG apparatus was located.

Cortisol and subjective measures

Saliva samples were obtained using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Samples were obtained at four assessment points over a 165 min period, at respectively -5 min (T0), +60 min (T1), +120 min (T2), and +160 min (T3) with reference to capsule ingestion. Biochemical analysis of free cortisol in saliva was performed using a competitive electrochemiluminescence immunoassay (ECLIA, Elecsys 2010, Roche Diagnostics), as described elsewhere (Van Aken et al., 2003).

Self-reported mood (tension, fatigue, depression, anxiety, and activation) was rated on 100 mm visual analogue scales (VAS) at T0, T1, and T3. In addition, state anxiety (STAI-state: Spielberger, 1983) was measured at T0 and T3.

Emotional Stroop task

Stimuli consisted of photographs of eight actors (four female), each displaying a happy, a neutral and an angry expression (Ekman & Friesen, 1976; Lundqvist et al., 1998). An oval area centered on the face was extracted to remove the hair and non-facial contours. The pictures were equalized in luminance, and colored with a red, green or blue filter. Masking stimuli consisted of oval configurations of randomly cut and reassembled fragments of face stimuli (Van Honk et al., 1998) . The total stimulus set consisted of 72 target face stimuli (8 actors x 3 expressions x 3 colors) and 6 masks (2 different x 3 colors). Stimulus presentation and response logging were controlled using E-prime software and a serial voice response box and microphone (Psychology Software Tools, inc.). The emotional Stroop task was administered in four phases. Participants started with a practice block of nine trials in which only masks were presented. Next, they completed a masked version of the task of 72 randomized trials. Each trial started with a 750 ms fixation cross, followed by a very brief (16.7 ms, 2 frames at 120 Hz) exposure to a target face, which was replaced by a mask of the same color. Participants were instructed to name this color as fast as possible, and vocal response initiation triggered offset of the masks. New trials started after a random inter-trial interval of 2-4 seconds. The masked version was followed by an unmasked version of the task, which differed only in absence of the masks. Thus, the target stimuli remained visible until

registration of responses. To determine whether participants were capable of consciously perceiving the masked facial expressions, the final phase of the task consisted of an awareness check in which a subset of 48 masked faces (each actor and expression twice) was presented to the participants. The instructions explicitly stated that the stimuli consisted of briefly presented faces and participants were asked to indicate (if necessary by guessing) whether the emotional expression of these faces was happy, neutral, or angry by pressing the corresponding response button.

Responses during the masked and unmasked version of the emotional Stroop task were audio-recorded, and incorrect responses (1.3%) were excluded from the analyses. Reaction times outliers were filtered by using a < 200 ms and > 1300 ms cut-off, and subsequent removal of all RTs exceeding 2.5 SD from the individual participants' mean (per task and session). These trials were also excluded from the ERP analyses. Remaining latencies (89.7% of all trials) were averaged over the facial expression types for each task and condition. Statistical analyses were performed using separate repeated measures ANOVAs for the masked and the unmasked task version, with condition (placebo, cortisol) and stimulus emotion (angry, neutral, happy) as within-subject factors.

Electrophysiological recording and analyses

The electroencephalogram (EEG) was recorded from 19 scalp locations according to the international 10–20 system and referred on-line to C3/C4. An average earlobe reference was derived off-line. Vertical electro-oculogram (EOG) was recorded bipolarly from the supraorbital and the infraorbital ridge of the right eye, and horizontal EOG from the outer canthi of both eyes. The ground electrode was located at Fpz. EEG impedances were kept below 5 kΩ. The EEG and EOG signals were digitized at 500 Hz and segmented off-line (using Brain Vision Analyzer software, version 1.05, Brain Products GmbH, 1998–2004) into 1000 ms epochs, from 200 ms before to 800 ms after stimulus onset. Trials with incorrect responses and outlier reaction times were excluded from the analyses. Single trials were corrected for the effects of eye blinks and eye movements using a standard procedure (Gratton et al., 1983). Data were filtered digitally with a 0.1 Hz high-pass filter (24 dB/oct roll-off) and a 35 Hz low-pass filter (24 dB/oct). Artifact rejection was performed by removing epochs with activity below 0.50 μV for > 100 ms, amplitudes exceeding ±75 μV, a voltage step per sampling point > 50

μV, and an absolute difference between two values >100 μV. Because of many artifacts in either the F3/F4 or the occipito-temporal (OT) electrodes, artifact rejection and further processing was performed separately for the midline (Fz, Cz, Pz) and the OT electrodes (O1, O2, T5, T6) in order to include as many trials as possible in each analysis.

Separate averages were computed for happy, angry and neutral faces as a function of task (masked, unmasked). Six components (P2, N2 and P3 at midline electrodes, and N170, P1, and P2 at OT electrodes) were quantified from the individual participants' waveforms. Peak amplitudes of these components were identified automatically as local maximum relative to the 200 ms pre-stimulus baseline in defined latency ranges, with manual confirmation. At midline electrodes the P2 (100-250 ms) and N2 (175 -300 ms) amplitudes were time-locked to Cz, and P3 amplitude (275-500 ms) was time-locked to Pz (Picton et al., 2000). At OT electrodes N170 amplitude (110-190 ms) was identified at T5 and T6, and P1 (60 -140 ms) and P2 (180-260 ms) amplitudes at O1 and O2.

The influence of cortisol administration on subjective measures, salivary cortisol, emotional Stroop task performance, and ERP peak amplitudes were tested with repeated measures analyses of variance (ANOVAs) using the Statistical Package for the Social Sciences (SPSS 14.0, SPSS Inc., 1989–2005).[1] For the ERP measures, only results involving significant main or interaction effects including Emotion or Condition will be reported. All statistical analyses employed a two-tailed alpha of .05. Effect sizes of significant results are reported as proportion of explained variance (partial eta squared [η^2]). The Greenhouse-Geisser correction was used when appropriate (epsilon [ε]).

Results

Cortisol and subjective measures

Salivary cortisol (nmol/l) measures (see Table 5.2) were skewed and therefore log transformed before statistical analysis. The data of one participant were excluded from the cortisol analyses due to unreliable saliva measurements. The results of a 2 × 4

[1] In addition to the analyses reported below, repeated measures ANOVAs including the factor drug order (cortisol first session or second session) were performed to investigate possible order effects related to repeated administration of the emotional Stroop task. The results of these analyses showed no significant differences between the first and second session in the placebo or the cortisol condition, for either the color-naming latencies or the ERP amplitudes per electrode. Therefore, this factor was not further included in the analyses.

Table 5.2. Mean free salivary cortisol levels (nmol/l) after placebo and cortisol administration relative to time of capsule intake (t = 0)

Time (min)	Placebo		Cortisol	
	M	*SD*	*M*	*SD*
-5	9.9	3.5	9.9	3.7
+60***	8.3	2.8	270.6	211.0
+120***	7.4	2.6	206.5	189.6
+165***	6.6	2.4	142.7	157.6

Note: *** p <.001 placebo vs. cortisol.

repeated measures ANOVA with Condition (placebo, cortisol) and Time (T0, T1, T2, T3) yielded a significant main effect of Condition, $F(1,15) = 168.41$, $p = .000$, $\eta^2 = 0.92$, and Time, $F(3,45) = 45.08$, $p = .000$, $\eta^2 = 0.45$, as well as a significant interaction of Condition × Time, $F(3,45) = 63.72$, $p = .000$, $\eta^2 = 0.81$. Follow-up F-tests showed that, as expected, salivary cortisol levels did not differ between conditions before capsule intake (T0), $F(1,15) = .022$, $p = .88$), but were significantly increased after cortisol administration compared to placebo from one hour after capsule intake until the end of the experiment (T1: $F(1,15) = 117.53$, $p = .000$, $\eta^2 = 0.89$; T2: $F(1,15) = 149.87$, $p = .000$, $\eta^2 = 0.91$; T3: $F(1,15) = 106.46$, $p = .000$, $\eta^2 = 0.88$). Note that the emotional Stroop task was administered between T2 and T3 (i.e., between 2 and 2.5h after capsule intake).

To investigate effects of cortisol administration on subjective mood (data not shown) we conducted separate repeated measures ANOVAs with Condition (placebo, cortisol) × Time for STAI-state (T0, T3), and VAS tension, fatigue, depression, anxiety, and activation (T0, T1, T3). Results showed no significant main or interaction effects of Condition on any of the subjective mood measures, except for a trend of Condition on VAS activation ($F(1,16 = 4.42$, $p = .052$, $\eta^2 = 0.21$; all other Fs< 1.73, ps > .21, η^2s < 0.10). Follow up analyses revealed that reported activation was higher in the placebo compared to the cortisol condition before capsule intake (T0: $F(1,16) = 5.33$, $p = .035$, $\eta^2 = 0.25$; placebo: $M = 52.8$, $SD = 17.4$; cortisol: $M = 40.7$, $SD = 14.2$), but not after capsule intake (T1: $F(1,16) = 2.56$, $p = .13$, $\eta^2 = 0.14$; T3: $F(1,16) = 0.39$, $p = .54$, $\eta^2 = 0.02$), indicating that this effect was not due to cortisol administration.

Behavioral results

Awareness check

A paired samples t-test showed that the number of correct responses on the awareness check did not differ as a function of condition (placebo vs. cortisol). We

Table 5.3. Means (and SD) of color naming latencies (ms).

	Masked	Unmasked
Placebo		
Angry faces	661 (123)	702 (134)
Neutral faces	657 (106)	698 (105)
Happy faces	670 (119)	704 (143)
Cortisol		
Angry faces	649 (131)	669 (140)
Neutral faces	639 (128)	674 (144)
Happy faces	641 (119)	667 (138)

therefore pooled the data of the two separate measures together to provide a more reliable measure of awareness during emotional Stroop performance (see Putman et al., 2007b). For a binomial test with n = 96 and Π = .33, the individual cut-off score (p < .05) lies at 41 correct responses. Of the 17 participants, five participants scored 44 or more on the test, and results of the masked task will therefore be reported with and without these participants. The mean number of correct responses of the remaining 12 participants was 33.9 (SD = 2.9).

Reaction times

Repeated measures ANOVAs with Condition (placebo, cortisol) and Emotion (angry, happy, neutral) showed no significant effects on color-naming latencies in either the masked task version, all Fs < 1.25, ps > .28 (without the five participants that scored above chance level at the awareness check, all Fs < 1.9, ps > .19), or the unmasked task version, all Fs < 3.16, ps > .090. See Table 5.3.

ERP results masked task: Midline electrodes (Fz, Cz, Pz)

See Figure 5.1 for grand average ERPs at Fz, Cz, and Pz in the masked task.

P2 amplitude

The repeated measures ANOVA with Condition (placebo, cortisol), Emotion (angry, happy, neutral) and Electrode (Fz, Cz, Pz) showed a significant interaction of Condition × Emotion on the P2 amplitudes in the masked task version, $F(2,32) = 3.99$, $p = .028$, $\eta^2 = 0.20$. Follow-up testing to clarify the nature of this interaction revealed that the effect of Emotion was significant in the placebo condition, $F(2,32) = 4.81$, $p = .015$, $\eta^2 = 0.23$, but not in the cortisol condition, $F(2,32) = 0.68$, $p = .52$. In the placebo condition,

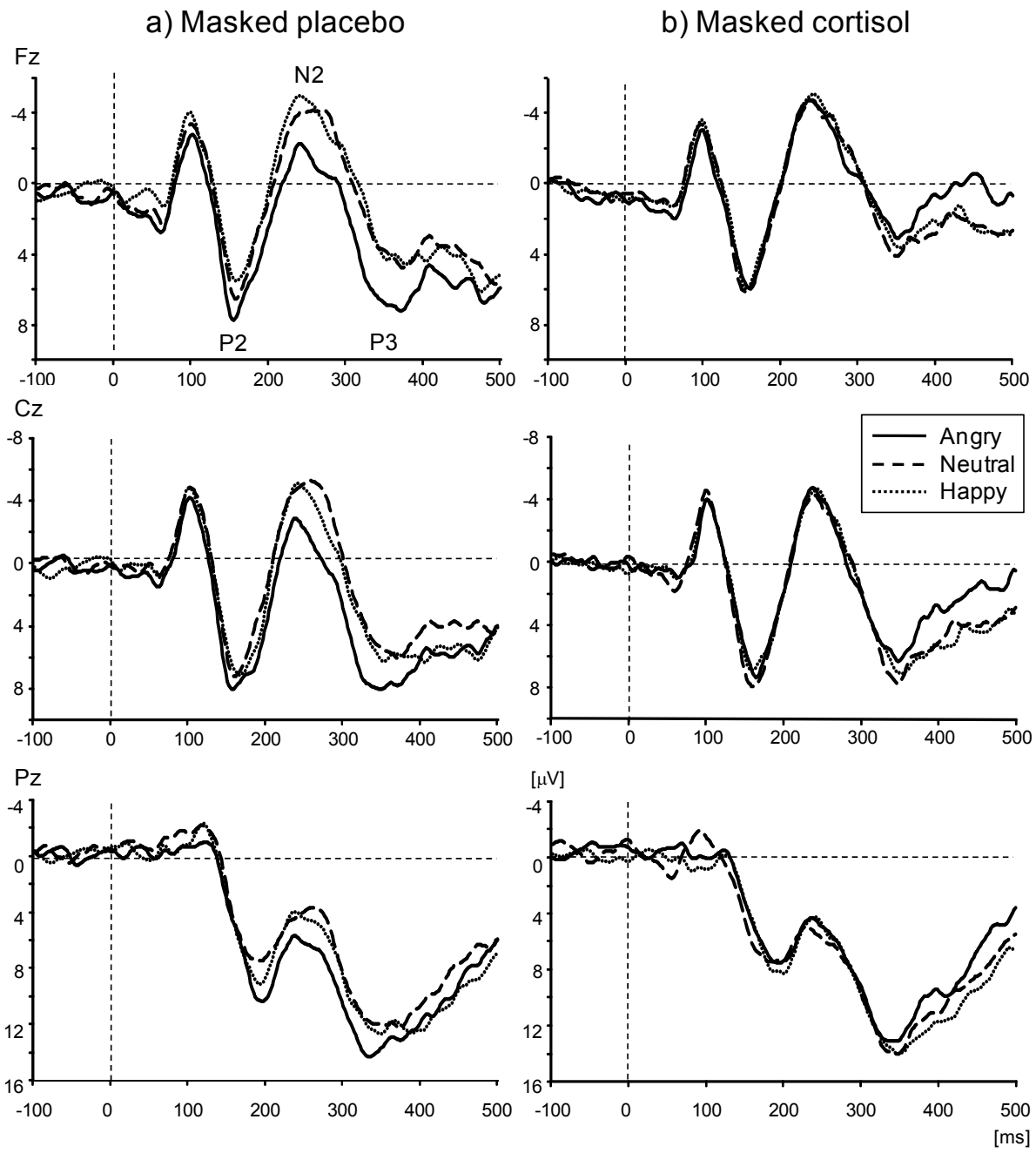

Figure 5.1. Stimulus synchronized grand average ERP waveforms at midline electrodes (Fz, Cz, Pz) in the masked emotional Stroop task after placebo (left) and cortisol (right) administration. Results showed a significant Condition x Emotion interaction at P2 and N2 components, reflecting significantly more positive amplitudes for angry faces in the placebo condition. This effect disappeared after cortisol administration.

the P2 amplitudes were significantly larger (more positive) for angry faces compared to both neutral, $F(1,16) = 7.93$, $p = .012$, $\eta^2 = 0.33$, and happy faces $F(1,16) = 4.60$, $p = .048$, $\eta^2 = 0.22$, but did not differ significantly between happy and neutral faces, $F(1,16) = 0.59$, $p = .46$). Cortisol administration tended to decrease the P2 amplitudes compared to placebo for angry faces, $F(1,16) = 3.88$, $p = .066$, $\eta^2 = 0.20$, but did not significantly affect

the P2 amplitudes for neutral, $F(1,16) = 1.91$, $p = .19$, or happy faces, $F(1,16) = 0.15$, $p = .71$. No other effects reached significance, all Fs < 2.2, ps > .12. The Condition × Emotion interaction remained significant when the participants that scored above chance level on the awareness check (n =5) were excluded from the analyses, $F(2,22) = 3.51$, $p = .048$, $\eta^2 = 0.24$. The P2 amplitudes for angry faces in the placebo condition remained significantly more positive compared to happy, $F(1,11) = 5.42$, $p = .040$, $\eta^2 = 0.33$, and neutral faces, $F(1,11) = 5.11$, $p = .045$, $\eta^2 = 0.32$. In addition, the decrease in P2 amplitudes after cortisol administration compared to placebo for angry faces was now significant, $F(1,11) = 7.31$, $p = .021$, $\eta^2 = 0.40$.

N2 amplitude

The results of the N2 amplitudes in the masked task version showed a significant main effect of Emotion, $F(2,32) = 3.81$, $p = .048$, $\varepsilon = 0.74$, $\eta^2 = 0.19$, which was further qualified by a significant Condition × Emotion interaction, $F(2,32) = 3.97$, $p = .029$, $\eta^2 = 0.20$. In line with the results of the P2 amplitudes, the effect of Emotion was significant in the placebo condition, $F(2,32) = 6.92$, $p = .003$, $\eta^2 = 0.30$, but not after cortisol administration, $F(2,32) = 0.34$, $p = .71$. In the placebo condition, the N2 amplitudes for angry faces were significantly decreased (i.e., more positive) compared to both neutral, $F(1,16) = 8.85$, $p = .009$, $\eta^2 = 0.36$, and happy faces, $F(1,16) = 10.16$, $p = .006$, $\eta^2 = 0.39$. The difference in N2 amplitude for neutral compared to happy faces was not significant, $F(1,16) = 0.03$, $p = .87$. Cortisol administration resulted in significantly more negative N2 amplitudes compared to placebo for angry faces, $F(1,16) = 14.38$, $p = .002$, $\eta^2 = 0.47$, but did not affect the N2 amplitudes for neutral, $F(1,16) = 0.14$, $p = .71$) or happy faces, $F(1,16) = 0.17$, $p = .69$. No other effects including Emotion or Condition reached significance, all Fs < 3.1, ps > .07. The Condition × Emotion interaction remained significant when the participants that scored above chance level on the awareness check (n =5) were excluded from the analyses, $F(2,22) = 5.12$, $p = .015$, $\eta^2 = 0.32$. The N2 amplitudes for angry faces in the placebo condition remained significantly decreased (i.e., more positive) compared to neutral, $F(1,11) = 9.22$, $p = .011$, $\eta^2 = 0.46$, and happy faces, $F(1,11) = 10.08$, $p = .009$, $\eta^2 = 0.48$, and the effect of cortisol administration on the N2 amplitudes for angry faces also remained significant, $F(1,11) = 15.67$, $p = .002$, $\eta^2 = 0.59$.

Table 5.4. Means (and Standard Errors) of peak amplitudes (μV) at midline and occipitotemporal electrodes as a function of condition, task and stimulus emotion.

		Masked			Unmasked		
		Angry	Neutral	Happy	Angry	Neutral	Happy
Midline							
P2	placebo	10.1 (1.4)	8.0 (1.4)	8.4 (1.3)	14.1 (1.1)	11.3 (12.3)	12.3 (1.3)
	cortisol	8.2 (1.2)	9.0 (1.3)	8.8 (1.3)	13.2 (1.2)	12.5 (1.4)	12.5 (1.4)
N2	placebo	-0.9 (1.3)	-3.5 (1.3)	-3.4 (1.5)	2.7 (1.5)	0.8 (1.6)	0.1 (1.7)
	cortisol	-3.6 (1.3)	-3.2 (1.2)	-3.8 (1.2)	1.2 (1.5)	0.1 (1.4)	0.8 (1.4)
P3	placebo	11.8 (1.6)	10.5 (1.5)	10.7 (1.3)	14.2 (1.8)	12.5 (1.4)	14.3 (1.6)
	cortisol	8.9 (1.0)	10.3 (1.4)	10.2 (1.3)	12.0 (1.4)	11.6 (1.5)	12.0 (1.4)
Occipito-temporal							
N170	placebo	-3.0 (1.0)	-4.0 (1.0)	-3.5 (0.9)	-8.5 (1.6)	-8.6 (1.1)	-9.1 (1.4)
	cortisol	-3.3 (1.0)	-2.9 (1.1)	-3.6 (1.0)	-8.1 (1.3)	-7.7 (1.2)	-8.2 (1.2)
P1	placebo	7.4 (1.7)	7.0 (1.7)	7.7 (1.8)	6.4 (1.5)	6.4 (1.7)	6.9 (1.6)
	cortisol	8.1 (1.9)	8.1 (1.6)	8.9 (1.7)	6.7 (1.5)	7.5 (1.8)	7.4 (1.7)
P2	placebo	16.6 (2.1)	15.7 (1.8)	16.0 (1.9)	13.5 (1.7)	14.0 (1.7)	13.3 (1.7)
	cortisol	16.5 (1.8)	17.2 (1.9)	17.3 (1.7)	13.4 (1.6)	14.0 (1.7)	12.8 (1.6)

Note: Midline at Fz/Cz/Pz electrodes; Occipito-temporal: N170 at T5/T6 electrodes; P1 and P2 at O1/O2 electrodes

P3 amplitude

In contrast to the P2 and N2 amplitudes, the P3 amplitudes did not show significant effects for Emotion, $F(2,32) = 0.01$, $p = .99$, or Condition × Emotion, $F(2,32) = 2.34$, $p = .11$. Furthermore, no other effects including Emotion or Condition reached significance, all Fs < 3.8, ps > .05.

Occipito-temporal electrodes (T5, T6, O1, O2)

The repeated measures ANOVAs showed no significant results for the N170 amplitudes (T5, T6), all Fs < 3.2, ps > .05, or the occipital P2 amplitudes (O1, O2), all Fs < 2.3, ps > .12, in the masked task version. For the occipital P1 amplitudes results showed a significant Emotion × Electrode interaction, $F(2,32) = 6.89$, $p = .003$, $\eta^2 = 0.30$, reflecting a significant effect of Emotion at O2, $F(2,32) = 3.89$, $p = .031$, $\eta^2 = 0.20$, but this effect disappeared after the participants that scored above chance level at the awareness check were excluded from the analyses, $F(2,22) = 2.32$, $p = .12$. No other effects reached significance, all Fs < 3.7, ps > .07. See Table 5.4.

ERP results unmasked task: Midline electrodes (Fz, Cz, Pz)

See Table 5.4 for mean amplitudes at midline and occipito-temporal electrodes in the unmasked task.

P2 amplitude

In contrast to the results of the masked task, the interaction of Condition × Emotion on the midline P2 amplitudes was not significant in the unmasked task, $F(2,32) = 1.85$, $p = .17$. We did find a significant main effect of Emotion, $F(2,32) = 5.32$, $p = .010$, $\eta^2 = 0.25$. Follow-up *F*-tests showed that the P2 amplitudes were significantly increased for angry compared to neutral faces, $F(1,16) = 12.94$, $p = .002$, $\eta^2 = 0.45$, and showed a tendency in the same direction for angry compared to happy faces, $F(1,16) = 3.95$, $p = .064$, $\eta^2 = 0.20$. The difference between the P2 amplitudes for neutral and happy faces was not significant, $F(1,16) = 0.95$, $p = .34$. No other effects including Emotion or Condition reached significance, all *F*s < 1.2, *p*s > .34.

N2 amplitude

Results of the N2 amplitudes showed a significant Electrode × Emotion interaction, $F(4,64) = 7.09$, $p = .001$, $\varepsilon = 0.62$, $\eta^2 = 0.31$. Follow-up *F*-tests showed a significant effect of Emotion only at Pz, $F(2,32) = 6.05$, $p = .006$, $\eta^2 = 0.27$, reflecting significantly less negative amplitudes for angry compared to happy faces, $F(1,16) = 11.24$, $p = .004$, $\eta^2 = 0.41$, and a trend in the same direction for angry compared to neutral faces, $F(1,16) = 3.63$, $p = .075$, $\eta^2 = 0.27$. This result is in line with the findings for the masked task. No other effects including Emotion or Condition reached significance, all *F*s < 3.2, *p*s > .05.

P3 amplitude

In line with the findings of the masked task, the results for the unmasked task version showed no significant effects involving Emotion or Condition on the P3 amplitudes, all *F*s < 1.9, *p*s >.18.

Occipito-temporal electrodes (T5, T6, O1, O2)

In line with the masked task, results of the unmasked task version showed no significant effects on the N170 amplitudes (T5, T6), all *F*s < 4.4, *p*s > .05, or the occipital P2 amplitudes (O1, O2), *F*s < 2.2, *p*s > .16. The occipital P1 amplitudes did not show any significant results in the unmasked task either, all *F*s < 2.25, *p*s > 12.

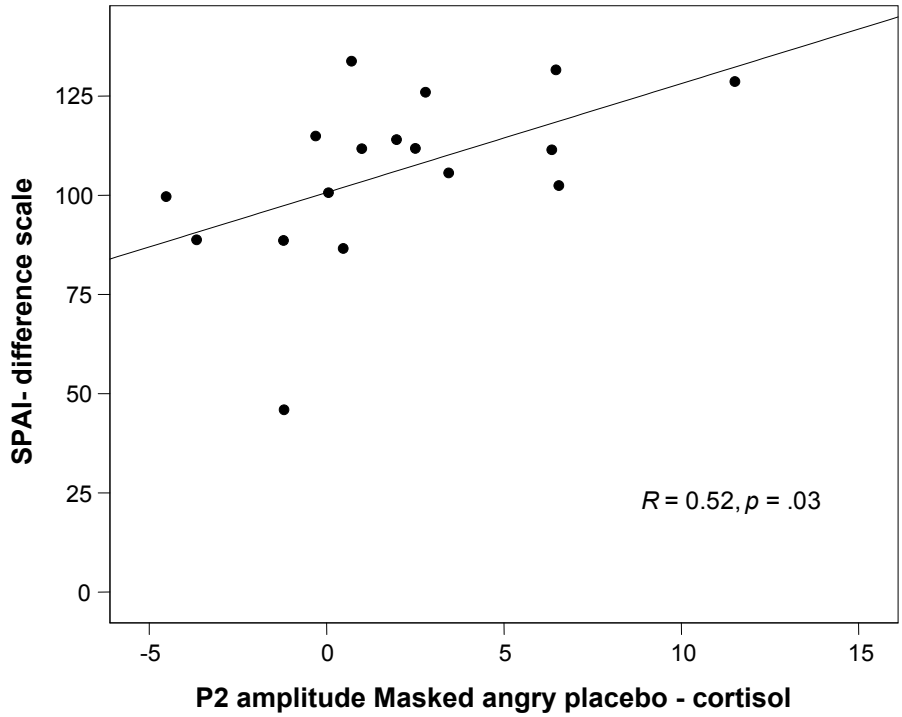

Figure 5.2. Correlation between social anxiety (SPAI total score) and cortisol-induced change in P2 amplitude for angry faces in the masked emotional Stroop task (i.e., P2 masked angry placebo – cortisol). Positive values on the x-axis indicate larger P2 amplitudes on midline electrodes (Fz, Cz, Pz) in the placebo condition, compared to the cortisol condition. The scatterplot shows that high levels of social anxiety are associated with a larger cortisol-induced decrease in P2 amplitudes for masked angry faces, indicating a decrease in threat processing (after cortisol administration) under conditions of restricted awareness.
Note: The correlation was somewhat depressed by one participant (see the lower left corner of the graph). Without this participant the correlation was $R = 0.56$, $p = .024$, but this person was no statistical outlier (Mahalanobis $D^2 = 8.64$, $p = .02$).

Relationship between threat processing and social anxiety

Since previous studies have shown significant relationships between ERP amplitudes during threat processing and severity of (social) anxiety (see Bar-Haim et al., 2005; Kolassa & Miltner, 2006), including effects of cortisol administration (Van Peer et al., 2009) we explored whether any of the significant ERP effects involving angry faces in the present study were influenced by individual differences in social anxiety. We investigated this by including social anxiety as a continuous factor in the repeated measures analyses (ANCOVA: see Judd et al., 2001) for the ERP amplitudes that showed a significant effect for angry faces. Significant interactions with social anxiety were followed up by calculating Pearson correlations between social anxiety and the difference score of the relevant within-subjects factor.

Results showed that the effect of Condition on the P2 amplitudes for angry faces in the masked task was significantly influenced by individual differences in social

anxiety, as reflected by a significant interaction of Condition with the SPAI total score, $F(1,15) = 5.63$, $p = .031$, $\eta^2 = 0.27$. Pearson correlations between total scores on the SPAI and the cortisol-induced change in P2 amplitude for masked angry faces (i.e., P2 amplitude masked angry placebo - cortisol) showed that the direction of this correlation was positive ($R = 0.52$, $p = .031$), indicating that patients with higher levels of social anxiety showed a significantly stronger decrease in P2 amplitudes after cortisol administration compared to placebo (see Figure 5.2). This effect was not significant for the SPAI social phobia scores, although the correlation showed the same direction of effects ($R = 0.33$, $p = .20$). None of the other ERP amplitude effects for angry faces in this study were significantly associated with individual differences in social anxiety.

Discussion

The major aims of the present study were to gain more insight in the temporal dynamics of biased processing of implicit social threat in SAD, and to explore the effect of cortisol administration on such processing. This was investigated by measuring ERPs during color-naming of masked and unmasked emotional faces in a modified emotional Stroop task after placebo and cortisol administration in participants with a clinical diagnosis of generalized SAD.

Early processing advantage for angry faces in SAD

First, the ERP results showed an early processing bias for social threat stimuli in the placebo condition, as reflected by increased (more positive) P2 amplitudes for angry compared to neutral and happy faces, even when they were presented under conditions of restricted awareness. ERP amplitudes for angry faces continued to be significantly more positive in the time-window of the N2 component on all electrodes in the masked task, as well as on Pz electrode in the unmasked task. Increased amplitudes of early positive frontocentral ERP components in reaction to threat-related (i.e., angry or fearful) emotional faces have previously been reported in studies in healthy participants, and are generally interpreted as reflecting increased allocation of processing resources to motivationally significant stimuli (Eimer & Holmes, 2002; Eimer et al., 2003; Bar-Haim et al., 2005; Williams et al., 2006).

To our knowledge, the present study is the first showing increased early

processing of implicit social threat reflected by ERPs in participants with a clinical diagnosis of SAD. This finding is in line with a range of behavioral studies showing hypervigilance to social threat in SAD (see Bar-Haim et al., 2007; Mobini & Grant 2007 for recent reviews), and provides support for the notion that such vigilance occurs in early stages of information processing (e.g., Mogg et al., 1997; Amir et al., 1998; Mogg & Bradley, 2002). ERP studies investigating angry face processing in relation to (social) anxiety are scarce, but there are some recent findings in non-clinical samples providing support for increased early threat processing in (socially) anxious participants. In line with our results, Bar-Haim et al. (2005) found enhanced P2 amplitudes to angry faces in high compared to low trait anxious healthy participants, indicating that implicit early threat processing can be modulated by anxiety (cf. Holmes et al., 2008; Moser et al., 2008). In addition, Rossignol, Anselme, Vermeulen, Philippot, and Campanella (2007) reported facilitated detection of subtle changes in anger expression during face repetition in participants with non-clinical social anxiety compared to low socially anxious participants, as reflected by a reduced N2b wave around 300 ms post-stimulus. Only a few previous ERP studies investigated angry face processing in participants with clinically diagnosed SAD (Kolassa & Miltner, 2006; Kolassa et al., 2007, 2009). In contrast to the present study, these found no evidence for increased early processing of task-irrelevant angry faces, although enhanced right temporo-parietal N170 amplitudes were found during explicit emotion identification of angry faces in one study (Kolassa & Miltner, 2006). The lack of a processing bias for implicit social threat in these studies may be partly due to the use of schematic face stimuli (Kolassa et al., 2007, 2009) which show a different electrophysiological response pattern than photographic faces (Kolassa et al., 2007) and may be less sensitive for detecting differential social threat processing in SAD. Furthermore, all of these studies (Kolassa & Miltner, 2006; Kolassa et al., 2007, 2009) focused on occipito-temporal electrodes and did not report on the early and late midline positive ERP components that we investigated in the present study, and which have consistently demonstrated emotional expression effects in healthy participants (see Holmes et al., 2008). Emotional modulation of the N170 component has been less consistently found and may be only present when the identification of the facial emotion is explicitly task-relevant, as suggested by Kolassa et al. (2009, see also Kolassa & Miltner, 2006), which would explain why we did not observe this effect in the present study.

Effects of cortisol on implicit threat processing

Our second main finding was that the early threat bias, reflected by increased P2 amplitudes for angry faces, significantly decreased (and disappeared) after cortisol administration in the masked task. Moreover, this effect was stronger for participants with higher levels of social anxiety. Both the finding that cortisol administration only significantly affected P2 amplitudes for angry faces, and the finding that the magnitude of this effect was related to severity of social anxiety are consistent with the results of Van Peer et al. (2009). However, the *direction* of the effect of cortisol administration in the present study was opposite to the findings of this previous study. Van Peer et al. (2009) investigated the effect of cortisol administration on ERPs in patients with SAD using a RT paradigm measuring social approach and avoidance behavior in reaction to happy and angry faces. In contrast to the *decrease* in P2 amplitudes for masked angry faces in the present study, the results of Van Peer et al. (2009) showed cortisol-induced *increases* in these same amplitudes, indicating enhanced processing, during avoidant responses to angry faces in patients with high levels of social anxiety (see also Van Peer et al., 2007). Notably, both of these tasks (i.e., the approach-avoidance task of Van Peer et al. (2009) and the emotional Stroop task described in the present article) were administered during the same experiment, and thus concerned the same group of participants. Therefore, the contrasting findings are most likely explained by task-related differences. First, cortisol only significantly decreased P2 amplitudes for *masked* angry faces in the present study, whereas stimuli in the previous study were all unmasked. Several authors have suggested that processing of unmasked threat stimuli may be affected by mood-controlling strategies in high socially anxious individuals (e.g., Williams et al., 1996; Mogg & Bradley, 2002), whereas such strategies are minimized during masked presentation. Consistent with this notion, behavioral evidence for modulation of angry face processing by social anxiety has been previously found in masked, but not unmasked facial emotional Stroop tasks (Putman et al., 2004). Similarly, angry face processing was significantly related to baseline endogenous cortisol levels in masked, but not unmasked versions of this task (Van Honk et al., 1998). Thus, masking has an important influence on the processing of these stimuli, and the preclusion of cognitive control processes could provide grounds for an interaction with cortisol.

A second important factor is that Van Peer et al. (2009) used an explicit affect-

evaluation task, whereas the emotional expression of the faces in the present task was implicit and task-irrelevant. Several studies suggest that task-relevance vs. irrelevance of emotional expression can have a significant effect on ERPs related to early threat processing (see e.g., Bar-Haim et al., 2005; Kolassa & Miltner, 2006; Eimer & Holmes, 2007; Kolassa et al., 2009). Furthermore, effects of cortisol on processing of negative or threatening emotional information may differ depending on task-relevance of these emotional stimuli. To our knowledge, only two previous studies investigated effects of cortisol administration on processing of task-irrelevant (or distracting) emotional stimuli. Putman et al. (2007b) reported increased color-naming latencies (reflecting interference or threat vigilance) for masked fearful compared to neutral faces on an emotional Stroop task after placebo administration. This effect was abolished after cortisol administration. Interestingly, this cortisol-induced decrease in fear processing was most pronounced in participants with high self-reported trait anxiety. More recently, Oei, Tollenaar, Spinhoven, and Elzinga (2009) found reduced interference by task-irrelevant negative pictures in a modified Sternberg working memory task after cortisol administration compared to placebo. Although both of these studies were conducted in healthy young men and did not use angry face stimuli, the pattern of results (reduced processing of task-irrelevant negative stimuli) is consistent with the present findings. In contrast, the findings of Van Peer et al. (2009) are in line with other studies showing increased angry face processing after cortisol administration in explicit emotion evaluation paradigms (Putman et al., 2007a; Van Peer et al., 2007, 2009). Thus, task or goal-relevance may be an important factor modulating the effects of corticosteroids on information processing, resulting in cortisol-induced increases in threat processing and avoidance when the stimuli are task-relevant, and inhibition of threat processing when the emotional stimuli are task irrelevant or distracting. These findings are consistent with the view that cortisol generally facilitates processing and adaptive behavior that is most relevant to the situation, as advocated by De Kloet et al. (1999) based on animal studies. Further research is needed to directly and systematically investigate the role of such task-related factors on the effect of cortisol on early attention processes.

The results of the present study suggest that social threat stimuli automatically attract more attention in patients with SAD at very early stages of information processing, and

that cortisol administration decreases this threat bias under conditions of restricted awareness. There are, however, some limitations that should be discussed.

First, we did not find any significant behavioral results, although this is in line with the results of other studies in patients with SAD using a similar paradigm (Kolassa & Miltner, 2006). Van Hooff, Dietz, Sharma, and Bowman (2008) suggested that a lack of behavioral findings in emotional Stroop studies with ERP could be due to the use of relatively long inter-trial intervals (ITIs, 2-4 seconds in the present study). However, previous behavioral studies using the same paradigm (with long ITIs) as the present study have shown it to be sensitive to detect attentional biases in high socially anxious healthy participants (see Putman et al., 2004), which makes this explanation less likely. A second possibility, as described in the introduction, is that behavioral results of the emotional Stroop task are less sensitive to detect attentional biases in social phobic patients, because RT data reflect the combined product of a range of cognitive processes, including possibly opposite biases in early and late stages of information processing. For this reason we included measurement of ERPs during task performance. Nevertheless, the demonstration of an attentional bias for threat using RTs would be helpful as a confirmatory measure to strengthen conclusions regarding ERP effects (e.g., Holmes, Bradley, Kragh Nielsen, & Mogg, 2009). Inclusion of a larger subject sample is also recommended to increase the statistical power to detect small or medium sized effects.

A second limitation is that we did not include a non-anxious control group, and therefore cannot conclude whether our finding of an early threat bias, or the effects of cortisol on this bias, are specific to social anxiety. The finding that effects of cortisol on early threat processing were stronger in patients with higher levels of social anxiety does provide tentative support for an increased sensitivity in high anxious patients, although this is limited by the fact that the participants are all within a restricted diagnostic range. In addition, a recent study by Putman et al. (2007b) using a highly similar Emotional Stroop task, showed a cortisol-induced decrease in fear processing that was most pronounced in healthy participants with high self-reported levels of trait anxiety. This finding supports the notion that anxious participants may show increased sensitivity to cortisol effects on threat processing compared to non-anxious participants (see also Roelofs et al., 2009a; Van Peer et al., 2007). Nevertheless, future ERP research including a matched healthy control group is necessary to conclude whether the effects of cortisol on implicit threat processing as found in the present study are specific to (or

increased in) socially anxious participants, or reflect a process that can be found in the general population.

Third, the masked task was administered prior to the unmasked task in all participants in order to minimize the chance that participants would consciously perceive the masked facial expressions due to, for example, priming effects. Although this does not affect our main findings, it may have confounded effects of masking with effects of repeated administration and should be accounted for in future studies.

Finally, in the present study we administered cortisol to investigate its causal influence on cognitive-emotional processes that play an important role in social anxiety disorder. Although exogenous administration studies are better suited to investigate the causal role of cortisol compared to e.g., stress induction, as they constrain effects related to arousal and noradrenergic activation, it should be noted that the results of these studies cannot simply be generalized to naturalistic situations with elevated cortisol levels. Thus, further research is needed to assess the ecological validity of our findings by comparing them with the effects of endogenous cortisol increases. In addition, the effects of cortisol in the present study relied on a single high (50 mg) dose, whereas dose-response studies in the field of memory research have shown an inverted U-shape relationship between cognition and glucocorticoid (GC) levels. That is, very high and low GC doses caused memory impairment, whereas moderate doses caused memory enhancement (see e.g., Lupien, Gillin, & Hauger, 1999). Future studies including more moderate doses of cortisol are needed to investigate whether different doses of cortisol result in a similar inverted U-shape effect on early threat processing.

To conclude, this study provided the first psychophysiological evidence for increased early processing of implicit social threat in participants with a clinical diagnosis of SAD, and showed that cortisol administration decreased this threat bias under conditions of restricted awareness. Together with previous findings (Van Peer et al., 2009) these results indicate a highly specific effect of cortisol on early motivated attention to social threat, and highlight the importance of motivational context (goal-relevance) and symptom severity.

Chapter 6

Affect-congruent approach and withdrawal movements of happy and angry faces facilitate affective categorization

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J. M. van Peer, M. Rotteveel, Ph. Spinhoven, M. S. Tollenaar, & K. Roelofs

Abstract

Increasing evidence indicates that evaluation of affective stimuli facilitates the execution of affect-congruent approach and avoidance responses, and vice versa. These effects are proposed to be mediated by increases or decreases in the relative distance to the stimulus, due to the participant's action. In a series of experiments we investigated whether stimulus categorization is similarly influenced when changes in this relative distance are due to movement of the stimulus instead of movements by the participant. Participants responded to happy and angry faces that appeared to approach (move towards) or withdraw (move away) from them. In line with previous findings, affective categorization was facilitated when the movement was congruent with stimulus valence, resulting in faster and more correct responses to approaching happy and withdrawing angry faces. These findings suggest that relative distance indeed plays a crucial role in approach-avoidance congruency effects, and that these effects do not depend on the execution of movements by the participant.

Introduction

Humans, like other animals, have a general tendency to avoid unpleasant, threatening stimuli and to approach pleasant stimuli (e.g., Chen & Bargh, 1999; Lang, Bradley, & Cuthbert, 1990). This link between affective evaluation and approach/avoidance behavior is supported by increasing evidence showing that the evaluation of stimulus valence facilitates affect-congruent approach and avoidance responses (i.e., relatively faster approach of positive and avoidance of negative stimuli (Chen & Bargh, 1999; Rotteveel & Phaf, 2004; Solarz, 1960). Conversely, the execution of various approach and avoidance related behaviors (i.e., pulling/pushing, nodding/shaking, smiling/frowning) can also influence stimulus evaluation, resulting in more positive evaluations when an approach movement is made and more negative evaluations when an avoidance movement is made (e.g., Cacioppo, Priester, & Berntson, 1993; see also Neumann, Förster, & Strack, 2003). Based on these findings, it has been proposed that the relation between affective stimulus evaluation and approach/avoidance behavior is bi-directional, and is most likely mediated by general approach and avoidance orientations (e.g., Neumann & Strack, 2000; Neumann et al., 2003).

Although it was initially thought (e.g., Cacioppo et al., 1993; Chen & Bargh, 1999; Rotteveel & Phaf, 2004) that these effects depended on a direct link between affect-evaluation and the execution of behavior (related to specific muscle activation), recent theories propose that this link is mediated by a more symbolic representation of experiences, which allows for more flexible processing and behavior as well as incorporation of task requirements (see e.g., Neumann et al., 2003; Niedenthal, Barsalou, Winkielman, Krauth-Gruber, & Ric, 2005; Strack & Deutsch, 2004). These models are supported by studies showing that congruency effects between affective stimuli and approach/avoidance responses are independent from specific physical (e.g., arm) movements. In these studies, approach and avoidance movements by the participant involve symbolic reduction of the distance between the affective stimulus and the participants' reference point, such as a manikin (De Houwer, Crombez, Baeyens, & Hermans, 2001) or the participants' name on a computer screen (Markman & Brendl, 2005). In addition, Neumann and Strack (2000, experiment 2) showed that visual cues inducing the impression that participants were moving toward or away from the

computer screen influenced the categorization of affective stimuli in a very similar way as the actual execution of approach and avoidance responses by the participant, suggesting that perception and action are similarly represented.

In all of the above studies, the approach and avoidance movements (whether actual, symbolic or suggested) were related to actions *by the participants themselves*. However, according to Neumann and Strack (2000) a crucial parameter of approach and avoidance is the regulation of the relative distance between an observer and an important object. Consequently, facilitation of affective categorization can similarly be expected for increases or decreases in the relative distance due to approach and avoidance movements *by the stimuli.*

The aim of the present study was to investigate whether changes in relative distance due to (perceived) approach and withdrawal movements of emotional stimuli facilitate the affective categorization of these stimuli in a similar way as affect-congruent movements executed by the participant himself. We used face stimuli in this study because faces convey important signals of threat or appeasement to humans (Bradley et al., 1997). Additionally, approaching and withdrawing faces may represent a natural phenomenon in social interaction. In line with the findings of Neumann and Strack (2000) as well as others (e.g., Chen & Bargh, 1999; Solarz, 1960) we predicted that the categorization of happy faces would be facilitated when they are perceived as approaching the participant, whereas the categorization of angry faces would be facilitated when they are perceived as withdrawing from the participant. In the first part of this study, we tested these predictions in three consecutive experiments, with different stimuli (schematic and photographic faces) and study samples (unselected participants and selected low and high trait avoidant participants).

In addition, we explored whether the predicted effects would hold when participants are asked to categorize the movement instead of the emotion of the stimuli. A recent study by Adams, Ambady, Macrea and Kleck (2006, study 1), using a similar manipulation of stimulus movement, showed an opposite pattern of results for angry faces (compared to our predictions above) when participants were instructed to categorize the *movement* instead of the *affective value* of the stimuli. In line with their prediction that angry expressions convey a behavioral intent to approach (i.e., aggression) by the actor, participants were faster to detect approaching compared to withdrawing angry faces. However, in contrast to their predictions a similar pattern of

results was found for fearful faces, raising the possibility that the facilitated detection of approaching angry faces in this study was related to the movement, rather than the emotional expression of the stimuli. Perhaps the instruction to attend to the movement instead of the affective value of the stimulus enhances the effect of movement, which in turn may override more subtle emotion effects. We tested this latter hypothesis in a fourth experiment.

In specific, in this article we present the results of four independent experiments: In the first experiment, based on the predictions by Neumann and Strack (2000), we investigated whether stimulus movement facilitated affective categorization of schematic drawings of an angry and a happy face. We then replicated and extended these findings in a second experiment, by investigating whether the strength of the effect for angry faces was influenced by individual differences in trait avoidance. In experiment 3, we replicated experiment 1 using photographic faces as stimuli, to exclude the possibility that the effects observed in the first experiments depended on physical characteristics of our stimulus material, rather than their emotional meaning. Finally, we conducted a fourth experiment to investigate whether the predicted congruency effects disappear when participants are instructed to categorize the movement instead of the affective value of the stimuli.

Experiment 1

Method

Participants

Twenty male students (age M = 21.5, SD = 2.8 years) recruited from Leiden University participated in the experiment for financial or course credit. These participants were recruited as a control group in the context of a larger study (see Tollenaar, Elzinga, Spinhoven, & Everaerd, 2009). All participants provided written informed consent prior to participation in the experiment, which was approved by the local ethical committee. The data of one participant were not recorded properly, resulting in a total of 19 participants.

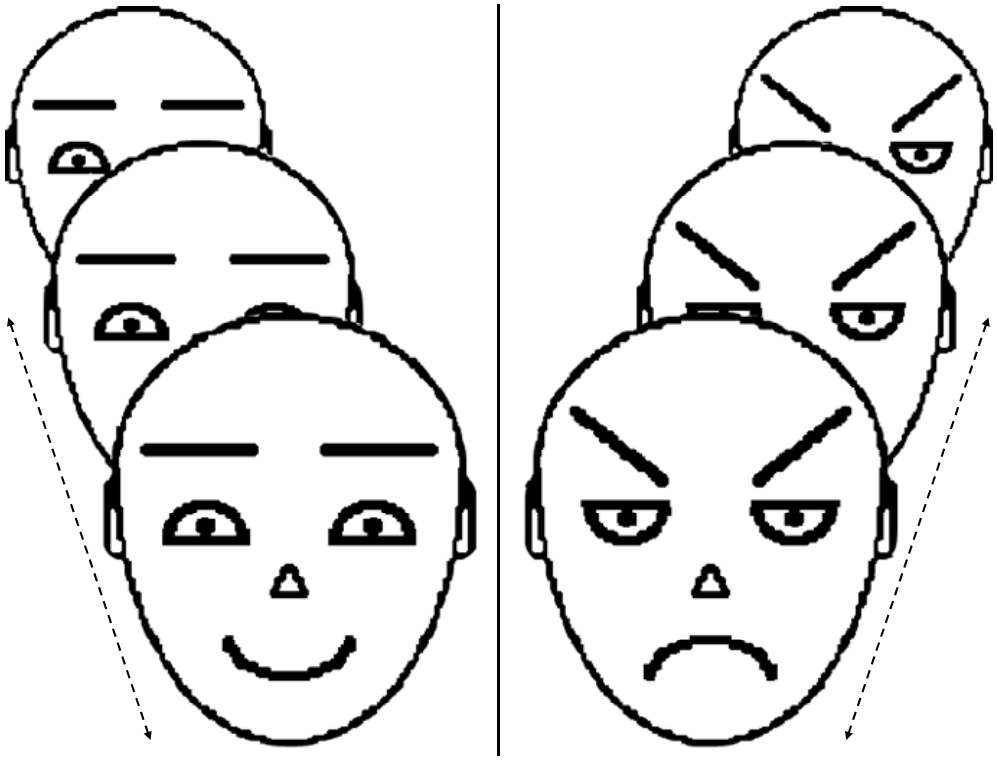

Figure 6.1. Example of stimuli used in Experiments 1 and 2, showing an enlarging happy and angry face to simulate approach. From Öhman, Lundqvist, & Esteves (2001, Figure 1). Copyright © 2001 by the American Psychological Association. Adapted with permission.

Materials and procedure

In this affective-evaluation task (Rotteveel, Bonarius, & Phaf, in preparation), schematic drawings of an angry (threatening) and a happy (friendly) face were presented (see Figure 6.1), consisting of modified versions of the faces presented by Öhman, Lundqvist, & Esteves (2001).[1] Sixteen pictures were created of each face by linearly reducing the size. The largest pictures measured 14 × 12 cm (*h* × *w*), the smallest pictures 5 × 4.5 cm. Pictures were presented in the center of a 15 inch computer screen at an 80 cm viewing distance.

Within a trial each picture was presented for 24 ms and replaced by the next picture in an order of either increasing or decreasing size, creating the illusion of an approaching or a withdrawing movement, respectively. The successive presentation of all 16 pictures was considered as one stimulus, resulting in a total stimulus presentation time of 400 ms. The time interval between successive stimuli was randomized between

[1] Preliminary research indicated that the frowned eyebrows of the original friendly face of Öhman et al. (2001) resulted in some emotional ambivalence, giving the face a sad appearance. Therefore, the friendly face was modified by replacing the frowned (/ \) eyebrows with straight eyebrows (- -). The new friendly face was perceived as having a more happy expression. The remaining facial features of the angry and happy faces were physically comparable. The size of the eyebrows was selected such that both faces contained an equal amount of pixels.

1500 and 2500 ms. All stimuli were presented in a semi randomized order (with a maximum of three happy or angry and three approaching or withdrawing stimuli consecutively) to each participant. Responses were given on two separate response buttons with the left and right index finger. Participants were instructed to indicate for each stimulus as fast and accurately as possible whether it had a happy or an angry facial expression.

The task consisted of two different response mapping conditions, in which the happy and angry faces were assigned to either the left or right response button. Both conditions were administered to each subject, separated by a short break (~ 5 min.). The order of conditions was completely counterbalanced across participants. Within each condition participants started with a practice block of 12 trials, followed by an experimental block of 80 trials.

Statistical analyses

The results of error rates and reaction times for correct responses (with RT between 150 and 1000 ms) were analyzed with separate 2 × 2 repeated measures analyses of variance (ANOVA rm) with stimulus Emotion (happy, angry) and stimulus Movement (approach, withdraw) as within subject factors. All statistical analyses employed a two-tailed alpha of .05. Residual (i.e. unexplained) variation is reported as Mean Square Error (MSE). As a rule, participants with extreme values on reaction times and error rates (*Z*-score > ± 2 on both measures) were excluded from the analyses, although no participants exceeded this criterion in the present experiment.

Results

Error rates

The error rates only showed a significant main effect of Emotion, $F(1,18) = 5.80$, $MSE = 8.86$, $p < .05$, indicating that participants made less errors when categorizing happy faces (3.8 %) than angry faces (5.5 %). No other effects were significant, all $F < 1.4$, $p > .25$.

Reaction times

The reaction times showed a significant Emotion × Movement interaction, $F(1,18) = 18.12$, $MSE = 130.83$, $p < .001$ (see Figure 6.2). In line with our expectations,

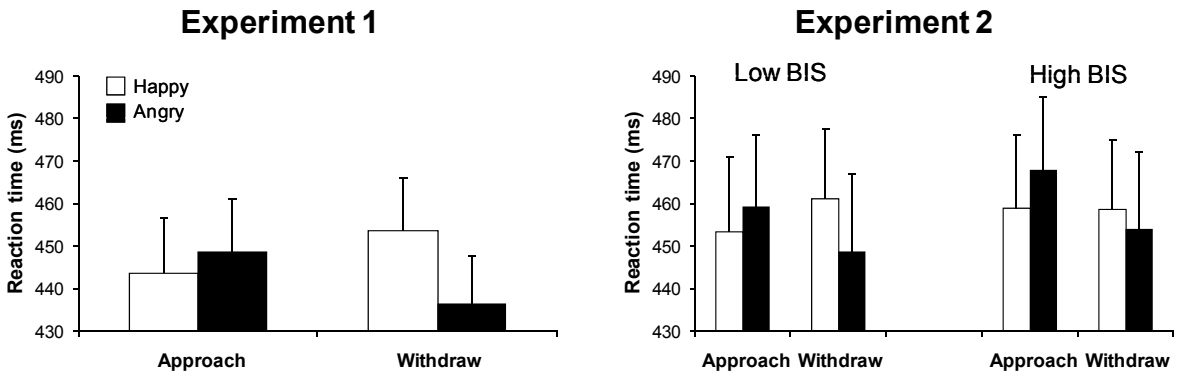

Figure 6.2. Mean (+ S.E.) reaction times (in ms) for correct responses to schematic faces (Experiments 1 and 2). Results show a significant interaction of Emotion x Movement in Experiment 1 (left, p<.001) as well as Experiment 2 (right, p<.001). This interaction did not differ significantly between low BIS and high BIS groups in experiment 2.

participants reacted faster to congruent stimuli (approaching happy and withdrawing angry faces) than to incongruent stimuli (approaching angry and withdrawing happy faces). Post hoc *F*-tests showed a significant effect of Movement for both happy, $F(1,18) = 5.51$, $MSE = 173.19$, $p < .05$, and angry faces, $F(1,18) = 6.41$, $MSE = 225.26$, $p < .05$, whereas the effect of Emotion was significant for withdrawal, $F(1,18) = 12.68$, $MSE = 226.48$, $p < .01$, but not for approach movements, $F(1,18) = 0.68$, $MSE = 341.71$, $p = .42$. The reaction times showed no other significant effects, all $F < 1.7$, $p > .20$.

Experiment 2

Experiment 1 showed that affect-congruent approach and withdrawal movements of happy and angry faces facilitated affective categorization of these faces. In a second experiment we aimed to replicate these findings and to investigate individual differences with respect to the strength of this effect. Neumann and Strack (2000) suggested that the link between affective stimulus evaluation and (perceived) execution of approach and avoidance responses is mediated by the activation of general approach or avoidance behavioral orientations within the participant (see also Chen & Bargh, 1999; Neumann et al., 2003). Several authors (e.g., Carver & White, 1994; Davidson, 1998; Gray, 1982; Roelofs, Elzinga, & Rotteveel, 2005; Roelofs et al., 2009a; Van Peer et al., 2007) propose that individuals differ in the relative activation of these behavioral orientations, which raises the question whether such individual differences would influence the strength of the congruency effects found in experiment 1. In experiment 2

we investigated whether individuals characterized by relatively high levels of trait avoidance show increased congruency effects for the angry faces compared to individuals characterized by low levels of trait avoidance.

Method

Participants

Forty male students (age *M* = 20.1, *SD* = 1.6 years) recruited from Leiden University participated in the experiment for financial or course credit. To investigate the effects of individual differences in trait avoidance on the reaction to approaching and withdrawing happy and angry stimuli, we selected a priori 20 students with low scores (≤ 16) and 20 students with high scores (≥ 21)[2] on the Behavioral Inhibition Scale (BIS). Individuals with high scores on this scale can be characterized as anxiety prone and tend to avoid threat (Carver & White, 1994). All participants provided written informed consent prior to participation in the experiment, which was approved by the local ethical committee.

Materials and procedure

Materials and task procedure were the same as in experiment 1, with one exception: Within each response mapping condition participants started with a practice block of 12 trials, followed by two experimental blocks of 60 trials each, separated by a short (~ 30 sec.) break. These data were collected as part of a control condition in the context of a larger study (see Van Peer et al., 2007; Van Peer, Roelofs, & Spinhoven, 2008). The results of error rates and reaction times for correct responses (with RT between 150 and 1000 ms) were analyzed with separate 2 × 2 × 2 repeated measures analyses of variance (ANOVA rm) with stimulus Emotion (happy, angry) and stimulus Movement (approach, withdraw) as within subject factors, and Group (low BIS, high BIS) as between-subjects factor. The data of one (high BIS) participant were excluded from the analyses because of extreme values on reaction times and error rates (*Z*-score > ± 2 on both measures), resulting in a total of 39 participants.

[2] Cutoff scores for these groups were based on the lower third and the upper third of the distribution of BIS scores (range 9-28, *M* = 18.5, *SD* = 3.6) in a sample of 153 male students.

Results

Error rates

Error rates were generally low (average < 6.3 %), and did not vary between groups or experimental conditions, all $F < 1.98$, $p > .16$.

Reaction times

The reaction times showed a significant main effect of Movement, $F(1,37) = 6.04$, $MSE = 157.17$, $p < .05$, indicating faster responses to withdrawing than to approaching faces. This main effect was qualified by the predicted interaction of Emotion × Movement, $F(1,37) = 11.05$, $MSE = 206.77$, $p < .01$ (see Figure 6.2), showing faster responses to congruent stimuli (approaching happy and withdrawing angry faces) than to incongruent stimuli (approaching angry and withdrawing happy faces).

Post hoc F-tests showed that the effect of Emotion was marginally significant for approach, $F(1,37) = 3.88$, $MSE = 218.94$, $p = .056$, and significant for withdrawal movements, $F(1,37) = 5.39$, $MSE = 274.43$, $p < .05$, whereas the effect of Movement was significant for angry, $F(1,37) = 17.20$, $MSE = 179.65$, $p < .001$, but not for happy faces, $F(1,37) = 0.78$, $MSE = 184.28$, $p = .38$.

We did not find any significant interactions between group and emotion in this task, all $F < 0.62$, $p > .44$.

Experiment 3

The results of the two experiments above showed that the categorization of schematic happy and angry faces was facilitated by apparent movement of these faces towards or away from the participant, respectively. However, the fact that in these experiments the emotionality of the stimuli was confounded with stimulus identity (i.e., we used only one stimulus face per emotion) raises the possibility that the observed effects are caused by the physical characteristics of the stimulus material, rather than their emotional meaning. To exclude this possibility we conducted a third experiment in which we replicated the first experiment, using happy and angry stimuli consisting of several different grayscale photographs.

Figure 6.3. Example of stimuli used in Experiments 3 and 4, showing an enlarging happy and angry face to simulate approach. From Lundqvist et al. (1998). Copyright © by the Section of Psychology, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden. Adapted with permission.

Method

Participants

Eighteen participants (15 female, age M = 21.4, SD = 1.8 years) participated in the experiment for financial or course credit. These participants were recruited from Leiden University for a battery consisting of several unrelated experiments.

Materials and procedure

The task procedure was the same as in experiment 1, with the following exceptions: Stimuli (see Figure 6.3) consisted of 20 grayscale photographs with happy and angry facial expressions (Lundqvist, Flykt, & Öhman, 1998). Both the happy and the angry expression were taken from the same models (total of 10 models, 50% female). The largest pictures measured 15.6 × 21.0 cm ($w \times h$), the smallest pictures 5.9 × 7.9 cm. Pictures were presented in the center of a 19 inch computer screen at an 80 cm viewing distance. Within a trial each picture was presented for 33 ms and replaced by the next picture in an order of either increasing or decreasing size (16 consecutive pictures), resulting in a total stimulus presentation time of 533 ms.

Each participant received (in counterbalanced order) two different response mapping conditions, in which the happy and angry expressions were assigned to either the left or right response button. Within each condition participants started with a practice block of 8 trials, followed by an experimental block of 80 trials. The stimuli used in the practice block (4 additional photographs: happy and angry face of 1 male and 1 female model) were not included in the experimental blocks.

Statistical analyses were the same as in experiment 1: Error rates and reaction times for correct responses were analyzed with separate 2 × 2 repeated measures analyses of variance (ANOVA rm) with stimulus Emotion (happy, angry) and stimulus Movement (approach, withdraw) as within subject factors. The data of one participant were excluded from the analyses because of extreme values on reaction times and error rates (*Z*-score > ± 2 on both measures), resulting in a total of 17 participants.

Results

Error rates

The error rates showed a significant main effect of Movement, $F(1,16) = 10.46$, $MSE = 10.16$, $p < .01$, suggesting that participants made less errors in response to approaching than withdrawing faces. However, this effect was modulated by a significant Emotion × Movement interaction, $F(1,16) = 10.54$, $MSE = 11.31$, $p < .01$ (see Figure 6.4A). Post hoc *F*-tests showed that, in line with expectations, participants made significantly less errors for approaching happy compared to angry faces, $F(1,16) = 4.63$, $MSE = 8.94$, $p < .05$, whereas this pattern was reversed for withdrawing faces, $F(1,16) = 8.13$, $MSE = 9.97$, $p < .05$. In addition, participants made significantly less errors for approaching compared to withdrawing happy faces, $F(1,16) = 14.99$, $MSE = 15.03$, $p < .001$, whereas the effect of Movement was not significant for angry faces, $F(1,16) = 0.03$, $MSE = 6.43$, $p = .87$.

Reaction times

Reaction times also showed a main effect of Movement, $F(1,16) = 11.99$, $MSE = 171.59$, $p < .01$, as well as a main effect of Emotion, $F(1,16) = 5.73$, $MSE = 783.33$, $p < .05$. Most importantly, in line with the error rates, these effects were modulated by a significant Emotion × Movement interaction, $F(1,16) = 5.01$, $MSE = 343.37$, $p < .05$ (see Figure 6.4B). Post hoc *F*-tests showed that, in line with expectations, participants

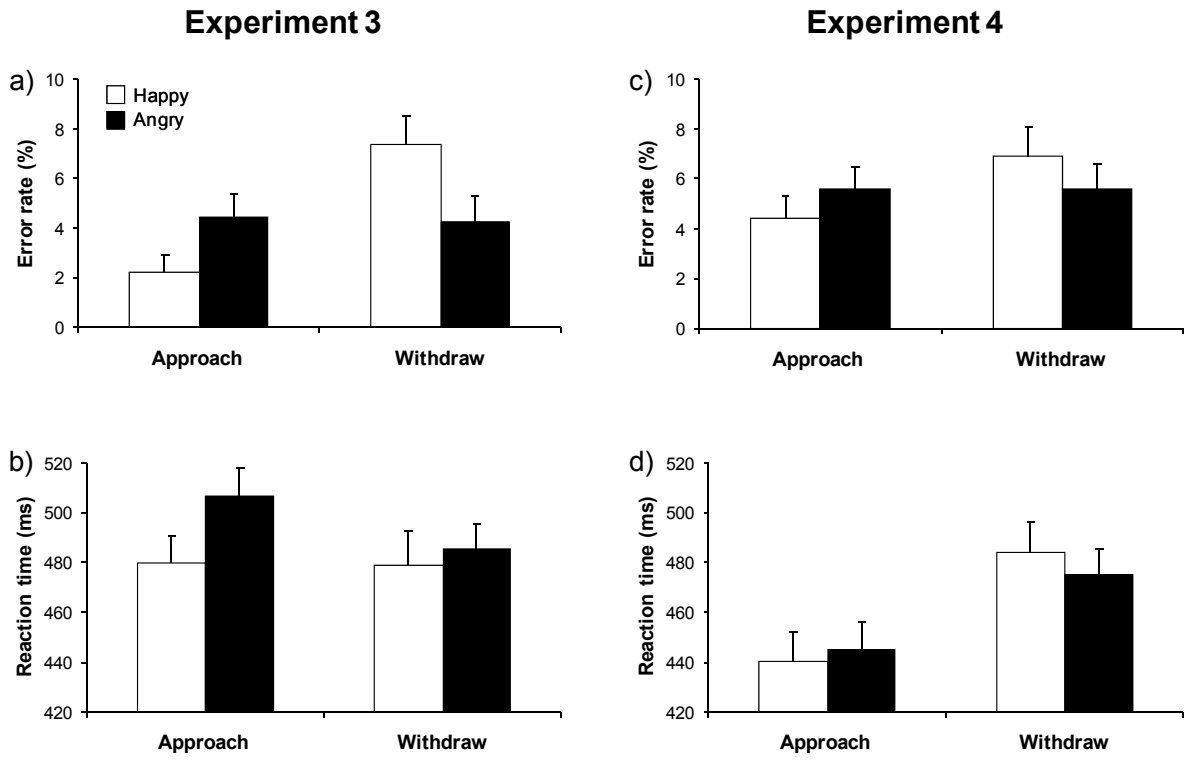

Figure 6.4. Mean (+ S.E.) error rates (in %) and reaction times (in ms) for correct responses to photographic faces (Experiments 3 and 4). Results show a significant interaction of Emotion x Movement in both the error rates (panel a) and the reaction times (panel b) of Experiment 3 ('judge emotion'). In contrast, this interaction was not significant in either the error rates (panel c) or reaction times (panel d) of Experiment 4 ('judge movement').

reacted significantly faster to approaching happy compared to angry faces, $F(1,16) = 9.88$, $MSE = 595.48$, $p < .01$, although the effect of emotion was not significant for withdrawal movements, $F(1,16) = 0.61$, $MSE = 531.23$, $p = .45$. In addition, participants reacted significantly faster to withdrawing compared to approaching angry faces, $F(1,16) = 14.84$, $MSE = 254.10$, $p < .001$, whereas the effect of Movement was not significant for happy faces, $F(1,16) = 0.03$, $MSE = 260.87$, $p = .87$.

Experiment 4

In line with expectations, both error rates and reaction times in experiment 3 showed the expected Emotion × Movement interaction. Finally, we conducted a fourth experiment to investigate whether a difference in response mode could explain the apparent conflict between our findings for angry faces and the findings of Adams et al. (2006). Materials and procedure of this experiment were exactly the same as in experiment 3, with the exception of the instruction. Instead of categorizing the stimulus

emotion, participants were (as in Adams et al., 2006, study 1) instructed to categorize stimulus movement (i.e., to indicate whether the stimuli were approaching them or withdrawing from them).

Participants

Twenty-three female participants (age M = 22.1, SD = 2.7 years) participated in the experiment for financial or course credit. These participants were recruited from Leiden University for a battery consisting of several unrelated experiments. The data of two participants were excluded from the analyses because of extreme values on reaction times and error rates (Z-score > ± 2 on both measures), resulting in a total of 21 participants.

Results

Error rates

The 2 × 2 stimulus Emotion (happy, angry) by stimulus Movement (approach, withdraw) repeated measures analysis of variance did not show any significant effects of Emotion or Movement in the error rates, all F < 2.04, p > .17. Most importantly, the Emotion × Movement interaction was not significant, $F(1,20)$ = 2.53, MSE = 12.97, p = .13, although participants did tend to make less errors for approaching compared to withdrawing happy faces, $F(1,20)$ = 3.89, MSE = 16.88, p = .063 (see Figure 6.4C).

Reaction times

In line with the error rates, the interaction of Emotion × Movement in the reaction times was not significant, although it showed a trend, $F(1,20)$ = 3.08, MSE = 320.89, p = .095, in the same direction as the results of our previous experiments (see Figure 6.4D). In addition, the reaction times showed a significant main effect of Movement, $F(1,20)$ = 53.24, MSE = 535.42, p < .001. Participants were significantly faster in reaction to approaching compared to withdrawing faces. Most importantly, this effect was not only significant for happy faces, $F(1,20)$ = 61.89, MSE = 324.00, p < .001, but, in marked contrast with the findings from our emotion evaluation experiments (experiment 1-3) also for angry faces, $F(1,20)$ = 17.73, MSE = 532.31, p < .001, as indicated by post hoc F-tests.

General discussion

Previous studies have shown that the execution of approach and avoidance responses affects stimulus evaluation, resulting in facilitation of positive evaluations when an approach movement is made and facilitation of negative evaluations when an avoidance movement is made by the participant (see e.g., Chen & Bargh, 1999; De Houwer et al., 2001; Markman & Brendl, 2005; Rotteveel & Phaf, 2004). Based on the assumption that regulation of the relative distance between the observer and the stimulus is a crucial parameter of these approach and avoidance effects (Neumann & Strack, 2000; see also Strack &Deutsch, 2004), we expected similar findings for increases or decreases in the relative distance due to approach and avoidance movements by the stimuli. In three experiments we investigated the effect of visual cues suggesting approach and withdrawal on affective categorization of happy and angry faces. In line with our predictions, the results of experiment 1 showed that the categorization of schematic happy faces was faster when these faces moved towards the participant, whereas categorization of schematic angry faces was faster when they moved away from the participant. The robustness of this effect was demonstrated by replication of these findings in two additional subject samples in experiment 2. The results of this second experiment further suggested that this affect-movement congruency effect was not influenced by individual differences in trait avoidance. Finally, we reproduced these congruency effects (in reaction times as well as error rates) in a third experiment using stimuli consisting of various photographic faces, which demonstrates that these effects do not depend on physical characteristics of the schematic faces used in the first two experiments.

As noted in the introduction, the findings for the angry faces in the present experiments are inconsistent with the findings of Adams et al. (2006, study 1), who investigated the effects of angry and fearful facial expressions on the categorization of approach and avoidance using a similar manipulation. In contrast to our findings, their results indicated faster reactions to approaching compared to withdrawing angry faces. However, the participants of Adams et al. (2006) were asked to categorize the movement and not (as in our experiments) the emotion of the stimuli. To investigate whether this methodological difference could cause the difference in findings, we conducted a fourth experiment in which we, like Adams et al. (2006, study 1) instructed

participants to categorize movement instead of emotion. Note that this was the only difference from experiment 3. Interestingly, the results of this fourth experiment did not show a significant interaction of Emotion × Movement, neither in the error rates nor in the reaction times. In line with the findings of Adams et al. (2006), and in marked contrast to experiment 3, in experiment 4 participants reacted significantly faster in response to approaching compared to withdrawing angry faces. We found the same pattern (i.e., faster responses to approaching stimuli) for happy faces. Notably, in contrast to their hypotheses, Adams et al. (2006) also found a similar pattern for fearful faces (although the significance of this effect was not reported). This raises the possibility that in these movement judgment tasks participants tend to be faster in judging movement of approaching than withdrawing stimuli in general, and that the observed effects for angry faces could be attributed to factors related to stimulus movement (e.g., physical stimulus characteristics or ease of categorization), rather than the emotional expression. Thus, the results of our experiment 4 demonstrate that a change of response instruction (judge movement instead of emotion) can reverse the reaction time pattern for angry faces, and can explain the discrepancy between our findings and those of Adams et al. (2006, study 1). A possible explanation for this effect could be that in these movement judgment tasks the saliency of the movement (which is explicit in this task) is so strong that congruency-effects of emotional expression (which is implicit in this task) are attenuated or overruled, resulting in a main effect of movement instead of an emotion by movement interaction. Another possibility is that when asked to judge the movement of an emotional face stimulus, participants focus more on the behavioral intent of the actor and less on their own behavioral tendency, which would reverse the effect for angry but not for happy faces. Thus, these findings suggest that the interaction between emotional expression and stimulus movement is complex and that besides the motivation of the participant, factors related to task goal (i.e., evaluation instruction) or the behavioral intent of the actor can influence the results. More research is needed to investigate the interplay of these different factors, preferably with different emotional expressions. For the moment, we suggest that conclusions with respect to the effects of angry expressions on judgment of behavioral intent in these movement judgment studies (Adams et al., 2006 as well as our experiment 4) should be drawn with caution, as long as an effect in the opposite

direction is not demonstrated for emotional expressions that are predicted to signal avoidance.

The findings of our first three experiments confirm the predictions of Neumann and Strack (2000) that perceived approach and avoidance movements of an object in the environment affect processing in a similar way as approach and avoidance movements executed by the participant. These findings fit well with recent theories suggesting that the link between approach/avoidance and evaluation is mediated by a symbolic representation of behavior, rather than concrete movements (see e.g., Neumann et al., 2003; Niedenthal et al., 2005). More specifically, they support the notion that approach and avoidance movements are coded in terms of relative distance between the participant's reference point and the evaluated object (Neumann & Strack, 2000; Strack & Deutsch, 2004). Previous support for this notion has come from findings showing that affect-movement congruency effects do not depend on the concrete motor actions performed by the participant, but on the outcome of these actions (i.e., relative distance increase or decrease) with respect to their reference point (De Houwer et al., 2001; Markman & Brendl, 2005; Seibt, Neumann, Nussinson, & Strack, 2008; Van Dantzig, Pecher, & Zwaan, 2008). Our experiments show that affect-congruent stimulus-movement is sufficient to reproduce these effects, and that they do not depend on the execution of a response by the participant. These findings are further supported by a recent study by Rotteveel and Phaf (2007, experiment 1), which showed that approach movements of affectively neutral word stimuli (Finnish words) tended to result in more positive responses towards these stimuli than avoidance movements. The present study is the first to show that both approach and avoidance movements of emotional faces facilitate movement-congruent emotion categorization.

An interesting remaining question is to what extent the present findings, as well as previously reported effects, are mediated by activation of approach and avoidance orientations. Neumann and Strack (2000) suggested that approach and avoidance movements activate approach and avoidance motivational orientations, which in turn facilitate affective processing. In the same way, processing of affective stimuli would activate these motivational orientations and subsequently facilitate affect-congruent responses (see also Neumann et al., 2003; Strack & Deutsch, 2004). Alternatively, these results may be explained by a more general affective stimulus-response compatibility

effect. For example, Eder and Rothermund (2008) suggested that affect-movement congruency effects are not restricted to typical approach and avoidance responses. In a comprehensive series of experiments they showed that affect-movement congruency effects could be replicated or reversed by using response (lever movement) labels (i.e., upwards, downwards) that had a positive or negative valence, but were intrinsically unrelated to approach and avoidance or distance reduction. Their evaluative response coding account explains these effects in terms of a general (mis-)match between stimulus valence and the valence (evaluative coding) of the response. Thus, this account provides a compelling explanation for the typical approach-avoidance congruency effects reported in previous studies, without drawing upon activation of motivational states. However, to explain the present findings according to this theory, we need to assume that the stimulus movements used in our tasks also have an evaluative valence (toward = positive, away = negative), which would consequently facilitate or interfere with the positive or negative response to the stimulus emotion (i.e., 'happy' or 'angry'). Although Eder and Rothermund (2008) did present evidence that the words 'toward' and 'away' are rated positively and negatively, respectively, more research is needed to investigate whether the same applies to stimulus movements as used in our tasks. In addition, an explanation is needed to clarify how the movements in our experiments would acquire this assumed valence. In the studies described by Eder and Rothermund (2008), the valence of the movement is assumed to depend on the labels used to describe the response in the task instructions. However, this explanation does not apply to our experiments, because here the stimulus movements are implicit (i.e., no reference is made to these movements in the task instructions). Thus, we suggest that more research is needed to investigate whether the present findings can be explained in terms of stimulus-response valence conflict, as suggested by the evaluative response coding view. Future studies incorporating neuroimaging techniques (e.g., fMRI) might shed more light on the question whether motivational or response-selection areas are particularly involved in these affective-congruency effects.

To conclude, the present experiments showed that movement direction of emotional faces (towards or away from the participant) differentially affects the speed of emotional evaluation of these stimuli, resulting in faster categorization of congruent (approaching happy and withdrawing angry faces) than incongruent (approaching angry and withdrawing happy faces) stimuli. These findings support current theories

suggesting that relative distance between a participant and the evaluated object plays an important role in approach-avoidance congruency effects. Moreover, they show that these effects do not depend on the execution of responses by the participant.

Chapter 7

Cortisol administration enhances the coupling of midfrontal delta and beta oscillations.

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International Journal of Psychophysiology (2008), 67, 144-150,
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J. M. van Peer, K. Roelofs, & Ph. Spinhoven

Abstract

There is increasing evidence that the strength of the relation between slow (SW) and fast (FW) wave activity in the EEG is associated with specific motivational states and their corresponding neuroendocrine patterns. Enhanced correlations between SW and FW have been related to anxiety, behavioral inhibition and high basal cortisol levels. However, the direct effects of cortisol on SW–FW coupling have not been experimentally studied yet. The present study investigated whether cortisol administration increases SW–FW coupling. Resting state EEG recordings were obtained from 40 right-handed healthy male participants with extreme low or high scores on a behavioral inhibition scale, after placebo and cortisol (50 mg) administration. As expected, delta–beta correlation tended to be higher in high compared to low behaviorally inhibited (BIS) participants. In addition, cortisol resulted in an increase in correlation between SW (delta) and FW (beta) activity compared to placebo, especially in high BIS participants. Consequently, the group differences were most pronounced after cortisol administration. These results suggest that cortisol can modify brain activity, increasing a pattern associated with anxiety and behavioral inhibition. This is in line with findings associating cortisol with behavioral inhibition and anxiety.

Introduction

There is increasing evidence that the strength of the relation between slow (SW) and fast (FW) wave activity in the EEG is associated with specific motivational states and their corresponding neuroendocrine patterns. Enhanced SW-FW correlations have been related to anxiety and behavioral inhibition (Knyazev & Slobodskaya, 2003; Knyazev et al., 2003; Knyazev, Savostyanov, & Levin, 2004; Knyazev, Schutter, & Van Honk, 2006), and were recently found in healthy participants with high basal cortisol levels (Schutter & Van Honk, 2005). This is an important finding, as high cortisol levels have been related to behavioral inhibition and anxiety (e.g., Korte, 2001; Sapolsky, 1990; Van Honk et al., 1998). Based on this finding, Schutter & Van Honk (2005) suggested that cortisol enhances SW-FW coupling. However, the direct effects of cortisol on SW-FW coupling have not been experimentally studied yet. In the present study we therefore investigated whether cortisol administration increases SW-FW coupling.

Slow and fast EEG waves are thought to be generated by separate neural systems with different functional properties (e.g., Başar, 2006; Başar, Başar-Eroğlu, Karakaş, & Schürmann, 2000; Klimesch, 1999; Laufs et al., 2003; Lopes da Silva, 1991). Although the specific brain regions involved in these systems and their specific functions are still not completely understood, some authors have suggested that SW (delta and theta) oscillations are EEG correlates of motivational and emotional processes associated with subcortical activity, whereas FW (alpha and beta) oscillations are correlates of more cognitive processes associated with cortical activity (e.g., Ray & Cole, 1985; Robinson, 1999, 2000, 2001; Knyazev, 2007; Knyazev & Slobodskaya, 2003; Neuper & Pfurtscheller, 2001).

Higher relative amplitudes of a given frequency in the EEG indicate more activity in the underlying system, which renders it more likely that a behavioral pattern associated with this system will occur. Relative SW and FW EEG spectral power may thus serve as a measure of predisposition to a specific behavioral style (Knyazev & Slobodskaya, 2003). Consistent with this proposition, measures of extraversion and behavioral activation have been found to be *positively* related to (SW) delta power, and *negatively* to (FW) alpha power. In contrast, neuroticism, behavioral inhibition and trait anxiety have been found to be *negatively* related to (SW) delta and theta power, and

positively to (FW) alpha and beta power (Knyazev, Slobodskaya, & Wilson, 2002; Knyazev et al., 2003, 2004).

Moreover, the relationship between SW and FW spectral power may provide insight into the balance or interaction of activity in the underlying systems. For example, relatively high SW-to-FW ratios have been associated with increased risk-taking behavior and impulsivity in healthy participants (Schutter & Van Honk, 2005) and patients with attention deficit hyperactivity disorder (see e.g., Loo, Hopfer, Teale, & Reite, 2004 for an overview). In addition, the strength of the reciprocal relationship between (FW) alpha and (SW) delta oscillations (in averaged evoked potentials as well as in resting state EEG) has been found to be *negatively* related to extraversion and behavioral activation (Knyazev et al., 2003; Robinson, 1999, 2001) and to be *positively* related to neuroticism, behavioral inhibition and trait anxiety (Knyazev & Slobodskaya, 2003; Knyazev et al., 2003, 2004, 2006). Similarly, experimental manipulation of anxious apprehension (by giving participants random negative feedback on their performance) has been found to significantly increase the correlation between (SW) delta and (FW) beta spectral power (i.e., delta-beta coupling) compared to baseline (pre-feedback) measurements (Knyazev et al., 2006).

Because these motivational states (i.e., behavioral inhibition and anxiety on the one hand and behavioral disinhibition or impulsivity on the other hand) have been associated with specific neuroendocrine patterns, Schutter and Van Honk (2004, 2005) hypothesized that the strength of the relationship between SW and FW activity may also vary as a function of these neuroendocrine patterns. The results of two studies supported this hypothesis: Administration of testosterone, a drug with clear disinhibitory and anxiolytic properties (e.g., Hermans, Putman, Baas, Koppeschaar, & Van Honk, 2006; Svensson, Akesson, Engel, & Soderpalm, 2003; Van Honk et al., 2004), to healthy volunteers was found to result in a decrease in the relation between delta and beta spectral power, resulting in a non-significant delta-beta correlation (i.e., delta-beta *de*coupling) (Schutter & Van Honk, 2004). In contrast, comparing the delta-beta correlation in healthy participants with high and low basal cortisol levels, Schutter and Van Honk (2005) found high cortisol levels to be associated with a significant delta-beta correlation (i.e., delta-beta coupling), whereas low cortisol levels were associated with the absence of delta-beta coupling. The latter finding is in agreement with findings associating the stress-hormone cortisol with behavioral inhibition and anxiety (e.g.,

Korte, 2001; Sapolsky, 1990; Van Honk et al., 1998) and suggests that cortisol can modify brain activity, increasing a pattern associated with anxiety and behavioral inhibition. Such finding supports the important influence of cortisol on cognition, emotion processing and behavior (e.g., De Kloet et al., 1999; Erickson, Drevets, & Schulkin, 2003). However, Schutter and Van Honk (2005) used a cross-sectional design and, as a result, the group differences between participants with high and low cortisol levels may be due to factors other than basal cortisol levels as well. To enable more conclusive interpretations of the effects of cortisol on the correlation between delta and beta activity, it needs to be shown whether cortisol *administration* can also increase this correlation.

The main purpose of the present study was to investigate whether cortisol can enhance delta-beta coupling by administration of cortisol in a within-subject, placebo-controlled design. In line with the findings of Schutter and Van Honk (2005), we expected to find an increased delta-beta correlation after cortisol compared to placebo. Second, since cortisol has been proposed as an endocrinological marker for behavioral inhibition and anxiety (e.g., Korte, 2001; Sapolsky, 1990; Van Honk et al., 1998), and since increased delta-beta coupling has been found in high anxious participants under conditions of uncertainty (Knyazev et al., 2006), we aimed to investigate whether high delta-beta coupling would also be associated with high behavioral inhibition.

Materials and Methods

Participants

Forty male students recruited from the University of Leiden participated in the study for financial or course credit. We selected a priori 20 students with low scores (≤ 16) and 20 students with high scores (≥ 21) on the Behavioral Inhibition Scale (BIS: Carver & White, 1994).[1] All participants were right-handed, had a bodyweight between 60-85 kg, and were screened to exclude any psychiatric disorder, clinical significant medical disease, past head injury with loss of consciousness > 5 min, and use of medication. All participants provided written informed consent prior to participation in

[1] Cutoff scores for these groups were based on the lower third and the upper third of the distribution of BIS scores (range 9-28, M = 18.5, SD = 3.6) in a sample of 153 male students.

the study, which was approved by the Medical Ethical Committee of the Leiden University Medical Center.

Procedure

The participants were given a 50 mg hydrocortisone or placebo capsule in a double-blind, within-subject crossover design. The order of cortisol or placebo administration was random and balanced within the high and low BIS groups. The two experimental sessions were one week apart. On the days of testing, participants arrived at the laboratory where, after a short introduction, drug administration took place at 12.30 or 14.30 h, followed by a resting period of one hour to allow for the cortisol to take effect.[2] During this period, participants completed questionnaires and the electrodes for the EEG measurements were placed. Subsequently, the experiment started with the measurement of resting state EEG, followed by a number of additional cognitive tests of which the results will be reported elsewhere (Van Peer et al., 2007). Resting state EEG was measured in a series of eight 1-minute recording periods, while the participants sat quietly with eyes opened and closed in counterbalanced trials (i.e., OCOCOCOC or COCOCOCO). With eyes opened, participants were instructed to look at a fixation point in front of them. Participants sat in a dimly lit, air-conditioned and sound-attenuated room, while the experimenter sat in an adjacent room where the EEG apparatus was located.

Psychophysiological data reduction and analysis

The electroencephalogram (EEG) was recorded from 19 scalp locations according to the international 10-20 system and referred on-line to C3/C4. Vertical electro-oculogram (EOG) was recorded bipolarly from the supraorbital and the infraorbital ridge of the right eye, and horizontal EOG from the outer canthi of both eyes. The ground electrode was located at Fpz. EEG impedances were kept below 5 kΩ. The EEG and EOG signals were digitized at 500 Hz. Signals were processed offline using Brain Vision Analyzer software (version 1.05, Brain Products GmbH, 1998-2004).

[2] Cortisol can readily cross the blood-brain barrier (see e.g. Herbert et al., 2006 for a review), and cortisol administration has been shown to significantly affect cognitive and emotional processes (e.g. Kirschbaum et al., 1996; Lupien et al., 1999; van Peer et al., 2007; Putman et al., 2007; Reuter, 2002) as well as resting state EEG (e.g. Tops et al., 2005; 2006) from one hour after administration, when cortisol levels reach their maximum (e.g. Reuter, 2002; see also van Peer et al., 2007).

Signals were re-referenced to an average-ears reference and filtered with Butterworth Zero Phase Filters (low cutoff: 0.1 Hz (12 dB/oct), high cutoff: 100 Hz (12 dB/oct), notch filter: 60 Hz). Subsequently, the data of each 1-minute recording period were segmented into 1.024 second segments, with 50% overlap. Rough artifact rejection (+/- 200 μV), followed by automatic ocular correction using a standard procedure (Gratton et al., 1983) and automatic artifact rejection (+/- 100 μV, lowest allowed activity 0.5 μV for 100 ms) were performed on these segments. The designation of an artifact in one of the leads resulted in the removal of that epoch for all channels in order to ensure that the remaining data were identical for all sites in time. A Fast Fourier Transform (FFT, full spectrum method, Hamming window of 10% length) was applied to chunks of artifact-free data to obtain estimates of spectral power density (μV^2/Hz). Spectral power density values for each electrode were averaged across all epochs within a single baseline and calculated for the delta (1-3 Hz), and beta (14-30 Hz) frequency bands. A natural log transformation (ln) was performed on these values to normalize the distribution. The data from one participant were excluded from the analyses because of deviant ln delta power density in both the placebo and cortisol condition (*Z*-score > 3.5 in both conditions).

Since a specific aim of the present study was to extend the findings of previous studies, we focused our primary analyses on the midfrontal (Fz) electrode, for which the effects on delta-beta correlation have been reported to be specifically pronounced (Schutter & Van Honk, 2004, 2005). However, in order to explore the topographic distribution of the effects of cortisol administration and group differences in delta-beta correlation, we performed additional analyses including more lateral and posterior electrodes (F3, F4, C3, Cz, C4, P3, Pz, P4).

Statistical analyses were performed on the Pearson correlation between ln average power density in the delta and beta frequency bands across all recording periods (i.e., the delta-beta correlation), at each electrode in each condition. To test for significant differences between correlations, we used Fisher's *r* to *r'* transformation to normalize the distribution of correlation coefficients, which allows the use of a *Z*-test to compare the correlations. To test the effect of cortisol administration we calculated a *Z*-score for non-independent groups, and to test for group differences we calculated a *Z*-score for independent groups (see Clark-Carter, 1997). Given our specific directional

hypotheses for increased delta-beta correlations associated with cortisol and trait inhibition, condition and group effects were tested with a one-tailed alpha of .05.

Salivary cortisol measures

To verify the effect of cortisol administration and to check for possible differences in baseline cortisol, saliva samples were obtained using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Samples were obtained before capsule intake and at the start of the EEG recording (one hour after capsule ingestion). Biochemical analysis of free cortisol in saliva was performed using a competitive electrochemiluminescence immunoassay (ECLIA, Elecsys 2010, Roche Diagnostics), as described elsewhere (Van Aken et al., 2003).

Results

Salivary cortisol

Salivary cortisol (nmol/L) measures were skewed and therefore natural log transformed before statistical analysis. As expected, unbound levels of cortisol did not differ between conditions before capsule intake (placebo: M = 9.1, SD = 3.0; cortisol: M = 9.4, SD = 2.8; t (37) = - 0.74, p = .47), but were significantly increased (t (37) = - 18.69, p < .001) one hour after cortisol administration (M = 173.4, SD = 142.3) compared to placebo (M = 6.8, SD = 1.7). There were no significant differences in salivary cortisol values between the low BIS and high BIS participants (all p > .20).

Delta-beta correlation

Effect of cortisol administration

First, to test the hypothesis that cortisol administration can enhance midfrontal delta-beta coupling, we calculated the delta-beta correlation at Fz electrode after placebo and cortisol administration for all participants (see Table 7.1). In line with our expectations, cortisol administration resulted in a near-significant increase in the midfrontal delta-beta correlation compared to placebo (Z = -1.57, p = .058).

Extended analyses including more lateral and posterior electrodes (F3, F4, C3, C4, Cz, P3, P4, and Pz) showed that cortisol administration tended to increase the delta-beta correlation on all electrodes (except for a decrease at F4), but besides the midfrontal

Table 7.1. Delta-beta correlation (Pearson correlation between mean ln delta power density and mean ln beta power density at Fz electrode) and mean (SD) delta and beta activity (ln power density at Fz electrode) after placebo and cortisol administration for all subjects and for the low BIS and high BIS subgroups.

Measure	Placebo			Cortisol		
	All (n = 39)	LBIS (n = 19)	HBIS (n = 20)	All (n = 39)	LBIS (n = 19)	HBIS (n = 20)
Delta-beta correlation	R =.29	R =.08	R = .50*	R = .51**	R =.31	R = .70**
Ln delta power	2.52(0.3)	2.55(0.3)	2.48(0.2)	2.49(0.2)	2.51(0.2)	2.47(0.3)
Ln beta power	-.97(0.4)	-.94(0.4)	-.99(0.4)	-.99(0.5)	-.98(0.5)	-.01(0.5)

* p < .05, ** p < .001

(Fz) electrode this effect only approached significance at P3 (Z = -1.35, p = .09) (see Figure 7.1, panel A).

Interestingly, in contrast to the effects on the *correlation* between midfrontal delta and beta power, paired samples t-tests showed no differences between the placebo and cortisol conditions in either mean ln delta power (t (38) = 0.80, p = .43) or mean ln beta power (t (38) = 0.94, p = .36) at Fz (see Table 7.1), nor at any of the other electrodes.

Behavioral inhibition

To test the hypothesis that high behavioral inhibition is associated with increased midfrontal delta-beta coupling, the delta-beta correlation at Fz electrode was calculated separately for high and low BIS-groups in the placebo and cortisol conditions (see Table 7.1 and Figure 7.2). As expected, the midfrontal delta-beta correlation in the placebo condition was significant in the high BIS group, but not the low BIS group (Group difference: Z = - 1.33, p = .09). Although cortisol administration resulted in a general increase in midfrontal delta-beta correlation compared to placebo (see above), this correlation remained non-significant after cortisol administration in the low BIS group (see Table 7.1). The group difference was borderline significant after cortisol administration (Z = - 1.57, p = .058).

In line with the findings on Fz, results of the extended analyses (including F3, F4, C3, C4, Cz, P3, P4, and Pz) showed significant delta-beta correlations for high BIS, but not for low BIS participants on all electrodes in the cortisol condition, and on almost all electrodes (except for a lower correlation at F3, F4, and C4) in the placebo condition (see Figure 7.1). However, besides the effect at Fz, in both conditions the group difference

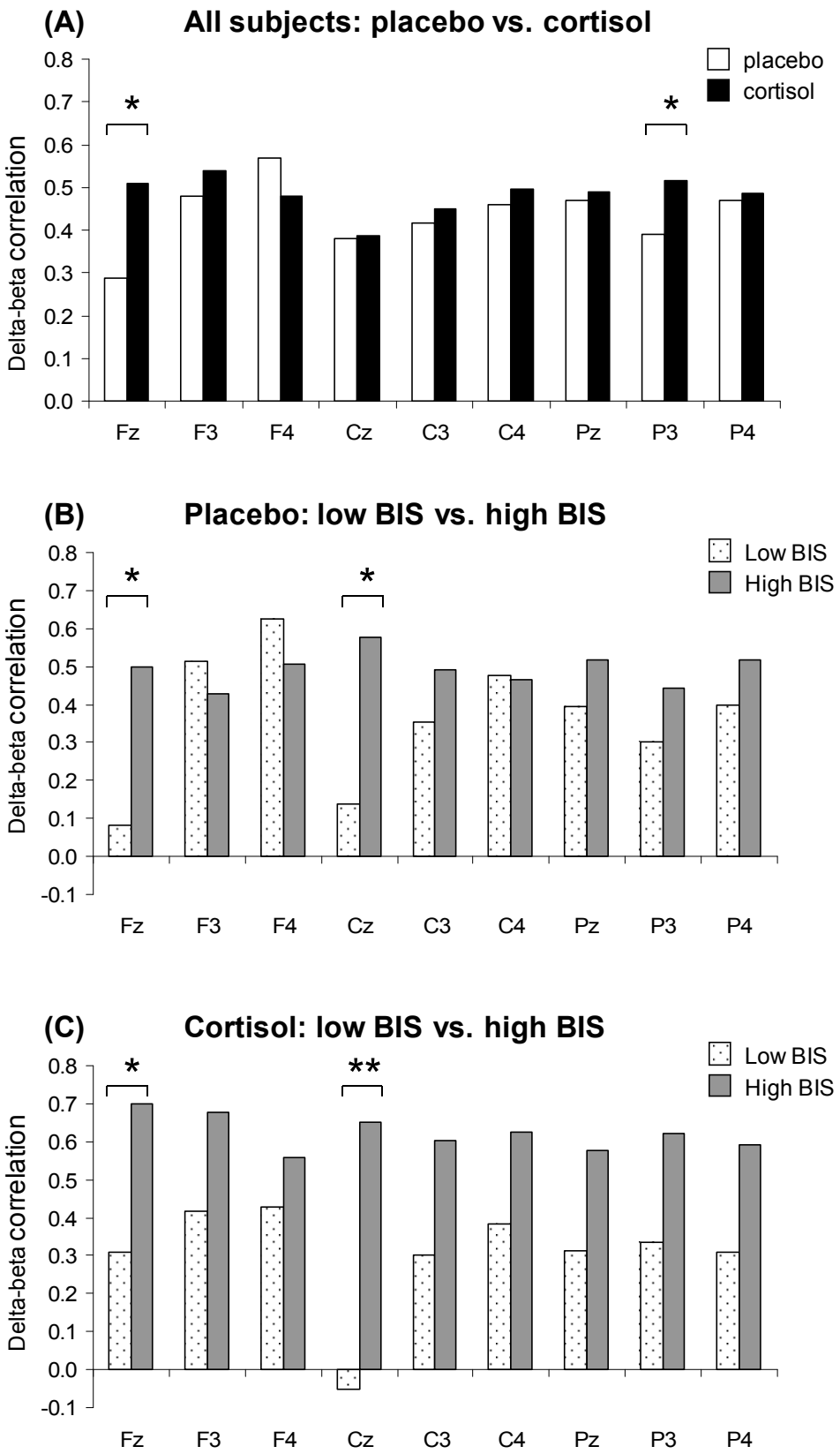

Figure 7.1. Delta-beta correlations (Pearson correlation between mean ln delta power density and mean ln beta power density) by electrode site for (A) all participants (n = 39) after placebo and cortisol administration, (B) low BIS (n = 19) and high BIS (n = 20) participants after placebo administration, and (C) low BIS and high BIS participants after cortisol administration. *$p < .10$ **$p < .05$ (1-tailed)

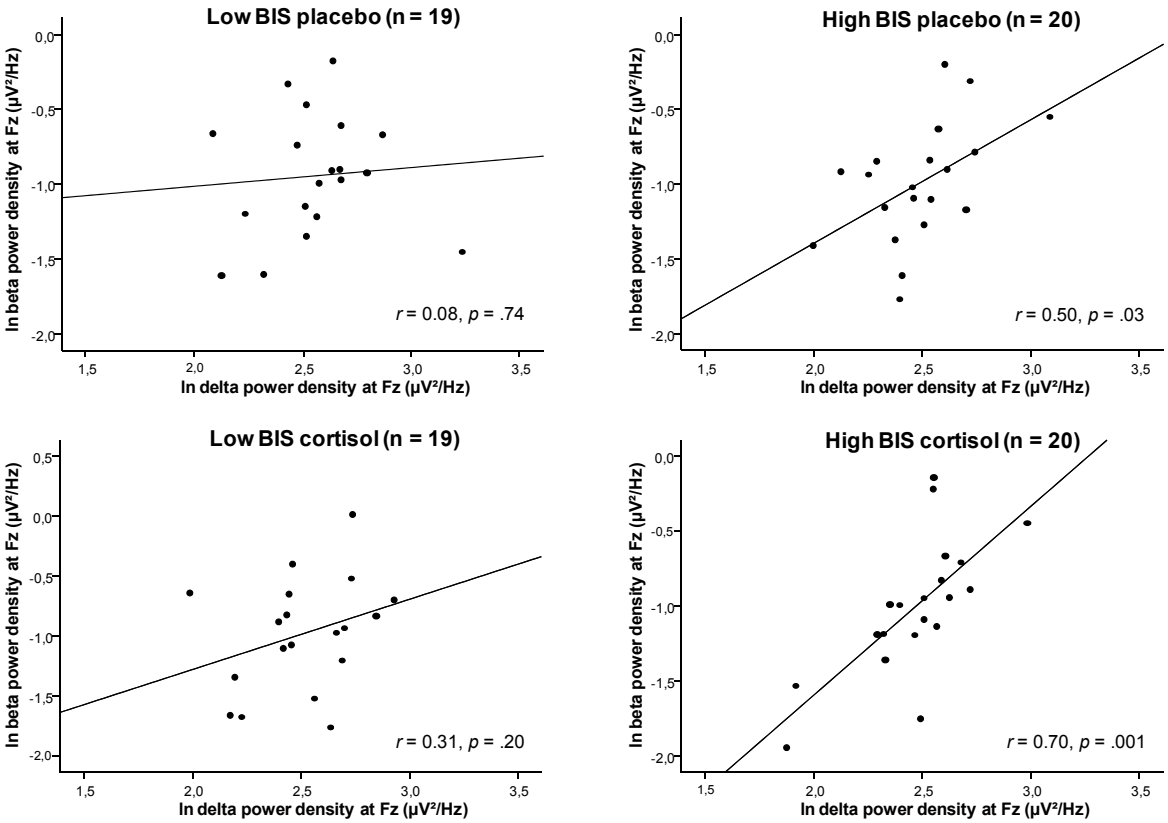

Figure 7.2. Significant midfrontal delta-beta correlation in high BIS participants (right), but not in low BIS participants (left) after placebo and cortisol administration. Cortisol administration (bottom) resulted in a general increase in delta-beta correlation compared to placebo (top) for all participants.

only approached significance at Cz (placebo: Z = -1.49, p = .07; cortisol: Z = -2.38, p <.01) (see Figure 7.1, panels B and C).

High and low BIS participants did not differ in either mean ln delta power (placebo: t (37) = 0.86, p = .39; cortisol: t (37) = 0.57, p = .57) or mean ln beta power (placebo: t (37) = 0.40, p = .69; cortisol: t (37) = 0.17, p = .87) at Fz (see Table 7.1), nor at any of the other electrodes.

Discussion

This study shows that cortisol administration tended to increase the correlation between midfrontal/central delta and beta spectral power in healthy male participants, especially in a group with high self-reported levels of behavioral inhibition, suggesting that cortisol can modulate brain activity, increasing a pattern that has been associated with anxiety and behavioral inhibition. These results replicate and extend previous findings of Schutter and Van Honk (2005) showing an increased correlation between

midfrontal delta and beta power in participants with high compared to low basal cortisol levels. The present study is the first to show that a within-subject experimental manipulation of cortisol can increase this correlation. These results make it unlikely that these differences can be attributed to other factors than cortisol.

Interestingly, cortisol administration only increased the *correlation* between midfrontal delta and beta power, and did not change mean (i.e., group) delta or beta power. This implicates that the increased correlation cannot be attributed to a general increase or decrease of activity in the neural systems underlying these oscillations (for example due to changes in arousal), and suggests that only the coherence between activity in the underlying systems is affected.

The present findings are important considering the increasing interest in the effects of cortisol administration on human emotion processing and behavior (e.g., De Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Putman et al., 2007a; Soravia et al., 2006; Tops et al., 2003, 2004; Van Peer et al., 2007). Despite this interest and though present brain states may influence subsequent processing (e.g., Başar, 2006), so far little was known about the effect of cortisol on brain activation associated with motivational states. Although studies investigating EEG frontal asymmetry (a widely studied index associated with the balance between approach and avoidance motivation, see e.g., Davidson, 1992) have shown a significant relationship between high endogenous cortisol levels and extreme right frontal asymmetry in primates (Kalin et al., 1998a) and infants (Buss et al., 2003), this relationship has not been reported for healthy human adults, and studies on the effects of acute cortisol administration in adults have produced mixed results (Tops et al., 2005, 2006). Given the now growing evidence showing a consistent relationship in healthy human adults between the strength of the midfrontal delta-beta correlation and different neuroendocrine patterns associated with motivational states (Schutter & Van Honk, 2004, 2005; present study) this measure can be considered a promising alternative or addition to the frontal asymmetry measure.

We also found significant differences in delta-beta correlation between participants with low and high scores on the Behavioral Inhibition Scale (Carver & White, 1994), especially at Cz after cortisol administration. The fact that the group difference was most pronounced after experimental manipulation of cortisol levels is consistent with findings of Knyazev et al. (2006), who reported a significantly higher delta-beta correlation in high trait anxious participants compared to low trait anxious

participants after an experimentally manipulated increase in uncertainty. These findings suggest that these group differences become more pronounced after experimental increase of anxious motivational states.

In the present study, the delta-beta correlation was highest in the high BIS group after cortisol administration. Interestingly, in a subsequently administered reaction time task measuring approach and avoidance tendencies to happy and angry faces, we found relatively facilitated avoidance reactions to angry faces only in these same high BIS participants after cortisol administration, as evidenced by relatively faster reaction times and increased positive event-related potentials when participants avoided an angry face (Van Peer et al., 2007). This suggests that high delta-beta correlations are associated with a reaction pattern related to social anxiety (e.g., Bögels & Mansell, 2004), though this needs direct confirmation.

All reported findings were most pronounced on the midfrontal and central electrode sites, confirming previous findings of Schutter and Van Honk (2004, 2005) suggesting that the midfrontal electrode site is most sensitive to neuroendocrine manipulation and individual differences in delta-beta correlation. These locations are in line with studies investigating SW-FW ratio in attention deficit hyperactivity disorder also reporting most pronounced differences at midline frontal and central electrode sites (Fz and Cz: see Lubar, Swartwood, Swartwood, & Timmermann, 1995).

Finally, it should be noted that although delta-beta correlations were strong and significant only in the high and not the low BIS group, especially after cortisol administration, these differences only approached statistical significance at midfrontal and central electrodes. This may be due to a lack of power in our study. Thus, studies including larger participants samples are needed to replicate our findings.

Based on theories associating SW with (subcortically driven) motivational or emotional processes and FW with cortically driven cognitive processes, several authors have suggested that enhanced SW-FW correlations reflect increased communication between subcortical and cortical brain regions, or even increased cortical control over subcortical drives (Knyazev & Slobodskaya, 2003; Robinson 2001; Schutter & Van Honk, 2005). Although such interpretation of the delta-beta correlation as an index of the information exchange between subcortical and cortical brain systems is still speculative, it fits with the findings of numerous direct and indirect connections between cortical and subcortical systems (e.g., Barbas, 2000), and is in agreement with the notion that

these connections play an important role in the regulation of emotion and behavior (e.g., LeDoux, 2000; Ochsner & Gross, 2005; Phelps, 2006). Hence, this interpretation may be used as a heuristic model, although

future studies are needed to further investigate the functional significance of the delta-beta correlation. For example, the identification of the functional neuroanatomical correlates of increases or decreases in delta-beta correlation (using functional Magnetic Resonance Imaging or Transcranial Magnetic Stimulation) may help to define the brain systems that relate to shifts in anxious/ inhibited or impulsive motivational states, and yield more insight in to what extent this correlation is related to inhibitory or excitatory connections. In addition, studies with within-subject measures of delta-beta correlation related to task performance may provide more insight into the relation between this motivational state and subsequent emotion processing and behavior.

In sum, the present study shows that cortisol administration tended to increase the correlation between SW (delta) and FW (beta) activity compared to placebo. In addition, delta-beta correlation was higher in high compared to low behaviorally inhibited subjects, and this group difference was most pronounced after cortisl administration. These results suggest that cortisol can modify brain activity, increasing a pattern associated with anxiety and behavioral inhibition. This finding is particularly interesting in the light of notions that cortisol prepares the organism for adaptive stress reactions and facilitates cognitive processes or behavior that is most relevant to the situation (e.g., De Kloet et al., 1999; Tops et al., 2006).

Chapter 8

General Discussion

The aim of this thesis was to gain more insight in the psychobiological mechanisms underlying social fear and avoidance behavior in humans. *Chapter 1* described that avoidance behavior may play an important role in social anxiety. However, experimental studies investigating the behavioral as well as neurobiological aspects of avoidance tendencies in SAD were largely lacking. In addition, whereas animal studies suggest that cortisol plays an important role in the regulation of social motivational behavior, little was known about the effects of cortisol on the regulation of avoidance behavior in humans, or even about effects of cortisol on attentional processing of threat. Thus, the aim of this thesis was to gain more insight in the brain processes underlying threat processing and avoidance behavior in high socially anxious individuals, and to investigate how these processes are affected by cortisol.

In this final chapter, I will first present a summary of the studies described in *Chapters 2* to *7* of this thesis, followed by an integration of the main findings and a discussion of the strengths and limitations of these studies. The chapter concludes with suggestions for future research and implications for clinical practice.

Overview of findings

In *Chapter 2*, the predictions were tested that individuals characterized by high levels of behavioral inhibition show preferential processing of and stronger avoidance tendencies towards social threat cues, and that these processes are facilitated by cortisol. This was investigated by measuring the effects of cortisol administration in a sample of pre-selected high and low behaviorally inhibited/anxious students, using a placebo controlled within-subject design. Overt approach and avoidance responses were assessed in reaction to positive and threatening social stimuli (i.e., happy and angry faces) using a reaction time affect-evaluation task (the approach–avoidance (AA-) task, Rotteveel & Phaf, 2004), and threat processing was measured by recording event-related potentials (ERPs) during task performance. The results of this study showed that cortisol administration significantly increased the AA congruency effects (i.e., relative faster avoidance compared to approach), in particular towards angry faces, in high but not low trait avoidant students. As reaction times for incongruent responses, such as approaching an angry face, reflect the costs of inhibiting an intuitive response tendency (i.e., to avoid the angry face) in favor of the instructed response (e.g., Roelofs et al.,

2009b), the increased AA congruency effect for angry faces is consistent with a relatively increased tendency to avoid threat. Moreover, the ERP results showed a significant effect of cortisol on social threat processing, again only in high trait avoidant participants. In these participants, cortisol administration resulted in a significant enhancement of processing of angry faces during avoidance behavior, as reflected by increased early (P150) and later (P3) midline positive ERP amplitudes. In line with our predictions, these findings suggest that cortisol administration results in a facilitation of processing and adaptive responses to motivationally significant threat stimuli, specifically in individuals that are highly sensitive to such threat cues.

The aim of the study presented in *Chapter 3* was to investigate the effects of endogenous cortisol increases on approach-avoidance behavior. Therefore, the Trier Social Stress Test (Kirschbaum et al., 1993) was administered to SAD patients, and performance on the AA-task in this psychosocial stress condition was compared to baseline using a within-subject design. The possible role of hypercortisolism in the failing regulation of social fear and fear behavior in SAD was investigated by directly relating stress-induced cortisol responses to overt avoidance responses to social threat cues. A sample of matched healthy participants and a sample of patients with Post-Traumatic Stress Disorder (PTSD) were included as control groups to investigate the specificity of the effects. This study showed three major findings: First, patients with SAD had larger cortisol responses to the social stress test as compared to healthy and PTSD control participants. Second, social stress elicited increased avoidance tendencies towards social threat stimuli in SAD, but not in PTSD patients and healthy controls. Third, the increased cortisol responses in the social stress condition were significantly correlated to the increase in social avoidance behavior in participants with SAD, over and above the effects of blood pressure and subjective anxiety. These findings provide evidence for a direct link between increased cortisol stress-responsiveness and social avoidance behavior in patients with SAD.

Following the study in *Chapter 3*, the study presented in *Chapter 4* was conducted to more closely investigate the causal role of cortisol, as well as the neural processes involved in the regulation of social fear behavior in SAD. Furthermore, the same experimental procedure was used as in *Chapter 2*, to test whether the findings for the high anxious students in that study would generalize to a clinical population. Therefore, in this study the effects of cortisol administration on approach-avoidance behavior and

threat processing were investigated in a second sample of unmedicated patients with SAD, using a placebo-controlled within-subject design. The results showed a significant positive relation between levels of social anxiety and slowing of approach movements to angry faces, consistent with a relatively increased tendency to avoid threat in more anxious patients. In contrast to the findings of *Chapters 2* and *3*, we did not find significant effects of cortisol on behavior in this study. However, and most importantly, the results of the ERP analyses demonstrated a significant interaction of cortisol by social anxiety on early processing of emotional faces: Cortisol administration resulted in a significant increase in processing of emotional faces during avoidance compared to approach for patients with high levels of social anxiety, as reflected by increased midline P150 amplitudes. This effect was only significant for angry faces, although it did not differ significantly for happy faces. These results are largely in line with the findings of *Chapter 2*, and suggest that the mechanism of early threat processing which is enhanced by cortisol in high trait avoidant healthy participants is similarly affected in patients with a clinical diagnosis of SAD. In addition, these findings suggest that the link between increased cortisol stress-responsiveness and social avoidance behavior in patients with SAD as found in *Chapter 3* may be mediated by causal effects of cortisol on early threat processing.

In *Chapter 5* the hypothesis was tested that patients with SAD show increased early processing of angry faces regardless of whether this is required for task performance, and even under conditions of restricted stimulus awareness. Furthermore, as effects of cortisol have been shown to be context-dependent, I investigated whether the cortisol-induced increases in threat processing, as found in previous chapters, are contingent on the stimuli being task-relevant. In this study, the effects of cortisol administration on RT and ERPs were measured in patients with SAD during color-naming of masked and unmasked emotional faces in a modified emotional Stroop task, using a placebo-controlled within-subject design. This study showed two major findings: First, the ERP results showed an early processing bias for masked as well as unmasked social threat stimuli, reflected by increased P2 amplitudes for angry faces in the placebo condition. Second, this early threat bias significantly decreased (and disappeared) after cortisol administration in the masked task, and this cortisol-induced decrease in threat processing was stronger for participants with higher levels of social anxiety. These results provide evidence that, in line with the predictions, social threat stimuli

automatically attract more attention in patients with SAD at very early stages of information processing. They further suggest that cortisol administration significantly decreases the processing of social threat stimuli when they are task-irrelevant and presented under conditions of restricted awareness, which is in contrast to the effect of cortisol on threat processing when stimulus evaluation is explicit and relevant for the generation of overt avoidance responses, as described in *Chapter 2* and *4*. I will further discuss the implications of these findings with respect to the context-dependency of cortisol effects in a separate paragraph below.

In *Chapter 6* a more theoretical-methodological issue was explored, namely to which extent the approach-avoidance effects, as measured in previous chapters, depend on the actions of the participants themselves or may be mediated by a representation of relative distance between the participant and the stimulus. In a series of four reaction time experiments in healthy male and female students, I tested the effects of stimulus movements on the evaluation of happy and angry face stimuli. It was predicted that changes in relative distance due to stimulus movement would influence affective evaluation in a similar way as the approach and avoidance movements executed by the participant in the AA-task. In line with these predictions, the results of these experiments showed facilitation of affective categorization of emotional faces when stimulus movement was congruent with stimulus emotion, resulting in faster and more correct responses to approaching happy and withdrawing angry faces compared to approaching angry and withdrawing happy faces. These findings suggest that relative distance indeed plays an important role in AA congruency effects, and that these effects do not depend on the execution of movements by the participant.

Finally, in *Chapter 7* the prediction was tested that cortisol administration increases the strength of the relation between slow (delta or theta) and fast wave (alpha or beta) activity in the EEG, a pattern of brain activity that has been related to high self-reported levels of behavioral inhibition and anxiety. Recently, Schutter and Van Honk (2005) found a significantly increased slow-fast wave (delta–beta) correlation in participants with high compared to low endogenous basal cortisol levels, and suggested that cortisol can modify brain activity, increasing a pattern associated with anxious and inhibited motivational states. However, the cross-sectional design of their study precluded conclusive interpretations regarding the causal effects of cortisol on the correlation between delta and beta activity. Therefore, in this chapter I investigated the

effects of cortisol *administration* on this resting state EEG measure in a sample of pre-selected high and low behaviorally inhibited/anxious students, using a placebo-controlled within-subject design. As expected, I found significant correlations between delta and beta activity at midfrontal electrodes in high but not low behaviorally inhibited subjects in both conditions. In addition, in line with the predictions, cortisol tended to increase the delta-beta correlation compared to placebo, resulting in significant delta-beta correlations on all electrodes in high but not low behaviorally inhibited participants, and a (near) significant group difference at midfrontal and central electrodes.

Theoretical integration

As described in *Chapter 1*, in this thesis I aimed to test several predictions concerning social threat processing and avoidance in social anxiety, and the role of cortisol in the regulation of these processes. In the next section, I will first discuss the results of the studies presented in *Chapters 2* to *7* in relation to these predictions, followed by another important theme that emerged from these studies, i.e., the context-dependency of cortisol effects on early threat processing.

Increased threat processing in social anxiety

The first prediction in this thesis was that socially threatening stimuli receive early preferential processing by high socially anxious individuals. This prediction was clearly supported by the findings in *Chapter 5*, in which we found increased early positive ERP amplitudes in reaction to angry faces even under conditions of restricted stimulus awareness, and while threat processing was not required for task performance. The fact that this threat vigilance was reflected in the ERPs but not in the behavioral results is consistent with the findings of others (e.g., Kolassa & Miltner, 2006), and underlines the added value of using this psychophysiological measure in emotional Stroop tasks. Two other studies also provided evidence for increased processing of social threat, during the execution of overt avoidance responses in high trait avoidant/behaviorally inhibited students (*Chapter 2*) and patients with high levels of clinical social anxiety (*Chapter 4*), but only after cortisol administration and not in the placebo condition. A possible reason for the absence of enhanced threat processing in

high anxious participants under baseline conditions (i.e., after placebo administration) in these latter studies could be that the approach-avoidance task in these studies involved the *explicit* evaluation of the emotional valence of the stimuli, and because the emotional valence was relevant for goal-directed social motivational behavior in this task (i.e., the execution or inhibition of overt approach and avoidance responses). This may have increased the motivational significance of all stimuli (positive as well as negative) for the participants, diminishing the relative processing advantages for social threat cues in high anxious individuals.

Increased avoidance tendencies in social anxiety

The second prediction of this thesis was that individuals characterized by high levels of behavioral inhibition or social anxiety would show stronger avoidance tendencies in reaction to social threat cues. Consistent with this prediction, the results described in Chapter 4 showed a significant effect of social anxiety on reaction times, reflecting slower execution of approach responses to angry faces in patients with higher levels of social anxiety. However, under baseline conditions (i.e., after placebo administration or before stress induction) we did not find significantly increased threat avoidance in high compared to low trait avoidant participants (*Chapter 2*) or in patients with SAD compared to patients with PTSD or healthy controls (*Chapter 3*). Although this lack of group differences in avoidance under baseline conditions is consistent with some previous findings on the AA-task (Roelofs et al., 2005), others authors have recently reported significant differences between low and high socially anxious participants on approach-avoidance behavior (Heuer et al., 2007). Several methodological differences may account for these findings. First, the participants in the study of Heuer et al. (2007) were pre-selected on extreme high and low scores (highest and lowest 10% of the distribution) on the Liebowitz Social Anxiety Scale (anxiety subscale). The healthy control participants in *Chapter 3* of this thesis were unselected, and although participants in *Chapter 2* were pre-selected on a behavioral inhibition scale (BIS: Carver & White, 1994), these groups were less extreme (highest and lowest 33% of the distribution). Second, the task used by Heuer and colleagues included a zoom feature, creating the visual impression that the pictures were coming closer upon pulling a joystick (approach) and that they moved away upon pushing it (avoidance). As shown in *Chapter 6* of this thesis, this zooming effect can by itself (i.e., independent from the

movement executed by the participant) result in significant approach-avoidance effects, and may thus be expected to increase these effects compared to a static stimulus as used in this thesis. Finally, in contrast to the AA-task used in this thesis, participants in the study of Heuer and colleagues responded to a neutral dimension (puzzles versus faces) instead of the stimulus emotion. Such indirect (implicit) measurement of the effects of emotional expressions has been argued to be more sensitive to individual differences in automatic processing and less affected by cognitive control mechanisms (e.g., De Houwer, 2006) compared to explicit evaluation. However, because of the zoom feature and the explicit instruction to the participants to pull the joystick towards themselves or push it away from themselves, the concepts of approach and avoidance are arguably not implicit in the AA-task of Heuer et al. (2007), whereas they are implicit in our task version.

Thus, we found evidence for increased avoidance tendencies in high anxious participants, sometimes at baseline but most evidently in a context of a (endogenous or exogenous) cortisol challenge. These findings not only provide experimental support for behavioral observations of avoidance tendencies in SAD (e.g., Alden & Bieling, 1998; Horley, Williams, Gonsalvez, & Gordon, 2003), but also shed light on the conditions in which these avoidance tendencies are most pronounced. This latter aspect will be discussed in the next section.

Cortisol facilitates social avoidance

The third and final main prediction in this thesis was that threat processing and avoidance would be facilitated by high levels of endogenous or exogenous cortisol. The findings of the studies in *Chapters 2*, *3* and *4* largely support this prediction. *Chapter 2* showed an increase in AA congruency effects after cortisol administration in high trait avoidant participants, consistent with a relatively increased avoidance tendency. In addition, the findings of *Chapter 3* demonstrated that social stress elicited increased avoidance tendencies toward social threat in patients with SAD, and moreover that this increased tendency to avoid threat was directly related to the stress-induced cortisol levels in these patients.

With regard to threat processing, *Chapters 2* and *4* provided evidence for facilitated processing of social threat stimuli in high behaviorally inhibited/anxious healthy participants (*Chapter 2*), as well as patients with high levels of clinical social

anxiety (*Chapter 4*). Interestingly, cortisol only increased threat processing during the execution of avoidance responses. This suggests that cortisol facilitates avoidance perhaps by facilitating motivated attention to threat.

By contrast, the findings of *Chapter 5* showed that cortisol administration significantly *decreased* processing of social threat stimuli when they were task-irrelevant and presented under conditions of restricted awareness. Thus, in line with the findings of *Chapter 4*, in *Chapter 5* cortisol administration specifically affected early positive ERP amplitudes for angry faces, and the magnitude of this effect was related to severity of social anxiety in both studies, but the *direction* of the cortisol effect was reversed. As both of these studies were conducted in the same participant sample and during the same experimental procedure, the contrasting findings are most likely explained by task-related differences, of which the task or goal-relevance of the stimuli may be an important factor (see *Chapter 5* for a more detailed discussion of task differences). Eimer and Holmes (2007) proposed that early midline positive ERP effects reflect higher order processing that is task dependent and relevant for the adaptive intentional control of behavior. As noted above, in the AA-task used in *Chapters 2* and *4*, evaluation of the emotional valence of the stimuli was explicit and relevant for goal-directed social motivational behavior. In contrast, in the emotional Stroop task in *Chapter 5*, the emotional valence of the stimuli was task-irrelevant and required inhibition, as the task goal was to quickly identify the stimulus color. Considering the above, the findings that cortisol administration facilitated processing of social threat stimuli during the execution of affect-congruent avoidance responses (*Chapters 2* and *4*), whereas it decreased processing of these stimuli when they interfered with the task goal (*Chapter 5*, see also Putman et al., 2007b; Oei et al., 2009), suggest that cortisol administration affects processing of motivationally significant threat stimuli in a goal-relevant and adaptive manner. This is consistent with the notion that glucocorticoid effects on cognition are generally adaptive, and facilitate behavior that is most relevant to the situation (De Kloet et al., 1999). In the next section, I will further discuss these findings in light of the view that the effects of cortisol on cognitive-emotional processing are context-dependent.

Context-dependency of cortisol effects

The results of the studies in this thesis in which we investigated the neural

processes associated with threat processing and avoidance behavior (*Chapters 2*, *4*, and *5*) demonstrated particularly robust effects of cortisol on early positive midline ERP components, which have been associated with "motivated attention", or facilitated processing of motivationally significant stimuli, possibly due to re-entrant projections from the amygdala to cortical regions (Amaral et al., 2003; Anderson & Phelps, 2001; Vuilleumier et al., 2004, see also *Chapter 1*). Furthermore, these results indicated that the effects of cortisol on this process are most pronounced for motivationally significant information (social threat cues), are stronger in individuals for which this information is highly relevant to their personal concerns (high trait avoidant or socially anxious participants), and depend on the relevance of the stimuli to the task goal. Together, these findings suggest that the effects of cortisol on cognitive-emotional processing are context-dependent, i.e., the strength and the direction of the effects depend on factors such as the processing stage, motivational significance and personal relevance of the stimuli, and task goal. This notion that effects of cortisol on cognitive-emotional processing are context-dependent is not new, and has been widely studied in the field of memory research. These studies have shown that the strength and direction of the effects of cortisol on memory processes (i.e., enhancement or impairment) depend, among other things, on factors such as the processing stage (encoding, consolidation, retrieval or reconsolidation), the emotional value (emotionally arousing versus neutral) and task-relevance (related or unrelated to the source of stress/emotional arousal) of the to-be-remembered material (see e.g., Lupien et al., 2007 for an extensive review). Furthermore, based on extensive animal research, De Kloet et al. (1999) proposed that corticosteroid effects on cognition are mediated by mineralocorticoid and glucocorticoid receptors in the brain, and depend on the relative activation of these receptors in the various stages of information processing. They further emphasized that the context in which this corticosteroid-receptor activation takes place is crucial, and that in general the effects of corticosteroids influence information-processing such as to increase adaptive behavior that is most relevant to the situation. The results of the studies in this thesis are generally consistent with this notion, but are the first to show such context-dependent effects of cortisol on early attentional processing of threat as measured by ERPs.

Strengths, limitations, and suggestions for future research

Strengths

First, we used a computerized RT paradigm to measure approach and avoidance responses in reaction to social stimuli, which ensured a direct and objective measure of behavior, and also makes it possible to measure brain activity during task performance. The studies presented in this thesis are the first using this paradigm for the investigation of approach and avoidance tendencies in a selected sample of patients with clinical SAD.

Second, we used ERPs to investigate the neural processes underlying threat processing and avoidance. ERPs are a highly sensitive measure of temporal dynamics of neural processing, and especially suitable for the study of very early attentional processes, which are much harder to measure with purely behavioral paradigms (see also Bar-Haim et al., 2005; Thomas et al., 2007).

Third, effects of cortisol on threat processing and behavior were investigated not only through experimental manipulation of endogenous cortisol levels (with a psychosocial stress task, *Chapter 3*) but also through acute administration of exogenous cortisol (*Chapters 2* and *4*). Exogenous administration was used in order to investigate the causal role of cortisol, because many factors interact with endogenous cortisol levels during stress-induction (e.g., arousal, social stress context, and individual differences).

Finally, in the studies presented in this thesis, we investigated threat processing and social avoidance behavior in high and low trait avoidant healthy students as well as in patients with a clinical diagnosis of social anxiety disorder. Studies in such pre-selected student samples can provide a valuable contribution to the understanding of basic processes implicated in anxiety disorders, but subsequent studies in clinical populations are necessary to draw conclusions about the generalizability of the findings and the clinical significance of these processes. Furthermore, we carefully selected participants based on strict criteria. In the studies in *Chapters 4* and *5*, patients using psychotropic medication were excluded, because this can significantly affect brain activity. We also excluded patients suffering from a concurrent major depression, as previous studies have shown that the attentional bias for threat in these patients may be moderated by the presence of a comorbid depression (Musa et al., 2003). A disadvantage of such strict selection criteria is that the patients in these studies may comprise an atypical representation of social anxiety patients in the general population, which

restricts the generalizability of the present findings. In *Chapter 3* patients with medication or depression were not excluded, but we controlled for these effects in the analyses.

Limitations

The studies presented in this thesis have several limitations, which are discussed in detail in the respective chapters. Here, I would like to focus on the limitations that are most important to the studies in this thesis in general.

The most important limitation is that we did not use a healthy control group for the clinical sample in the studies in *Chapters 4* and *5*. Although this does not limit the interpretation of the findings in these studies, a matched control group would have offered more information regarding the specificity of the effects of cortisol on threat processing for social anxiety. The within-subject design that was used for cortisol administration in these studies did allow us to control for individual differences in symptom severity, which proved to be an important moderating factor with regard to the effects of cortisol on threat processing. The effects of cortisol were stronger in patients with higher levels of social anxiety in these studies, which does point at an increased sensitivity in high anxious patients. This is consistent with other studies (*Chapters 2* and *3*; see also Putman et al., 2007b), but future studies including a matched control group are necessary to draw definite conclusions about such increased sensitivity to cortisol in patients.

Second, although the results of the studies in this thesis together suggest that the effects of cortisol on early threat processing are context-dependent, we did not investigate all aspects of this context-dependency in a controlled manner. We did control factors such as personal relevance and motivational significance of the stimuli, but studies in which factors such as goal-relevance are systematically manipulated are necessary to further investigate this issue.

Third, the external validity of the approach avoidance task for social behavior in real-life situations is thus far only supported by relations with self-report measures. However, using a highly similar paradigm, approach and avoidance tendencies towards spiders in spider phobic individuals have been shown to be reliably related to performance in a behavioral assessment test (BAT), in which participants were asked to approach a large living spider (Rinck & Becker, 2007). This provides general support for

the external validity of this paradigm in phobic individuals, but more research is needed to directly test associations of the social version of this task with real-life social avoidance.

Finally, in some studies (*Chapters 3* and *5*) we did not find an effect of cortisol on behavioral results, although we did find effects on ERPs. Future studies in which vigilance or avoidance of threat can be demonstrated using RTs would be helpful as a confirmatory measure to strengthen conclusions regarding ERP effects (e.g., Holmes et al., 2009), and to further investigate how increased early threat processing is associated with subsequent processing and behavior.

Suggestions for future research

In addition to the suggestions for future research mentioned above, I would like to outline a number of areas of specific interest for the study of cortisol effects on threat processing and avoidance.

First, studies using neuroimaging techniques such as fMRI should further investigate the brain areas that play a crucial role in the regulation of social motivational behavior in SAD, and how activity in the social motivational brain network is modulated by cortisol.

Second, it would be interesting to investigate the effects of cortisol on other measures of avoidance behavior, such as eye movements (see e.g., Horley et al., 2003) or social interactions, to see whether our findings on the approach-avoidance task generalize to other situations.

Third, in contrast to exogenous administration of cortisol, manipulation of endogenous cortisol levels through social stress induction results is associated with a number of concurrent stress-responses, such as noradrenergic activation, which may interact with the effects of cortisol on prefrontal function, as suggested by the results of several recent studies (e.g., Roozendaal et al., 2004; Elzinga & Roelofs, 2005). Future studies in which the effects of endogenous cortisol are attenuated, for example with the use of selective steroid receptor antagonists (e.g., metyrapone), may help to further assess the causal role of endogenous cortisol and the interplay with contextual effects in social avoidance behavior (De Kloet et al., 1999).

Fourth, future studies investigating the effects of cortisol on emotional or social motivational processing should systematically investigate the effects of context.

Finally, in contrast to most previous studies investigating the effects of cortisol on cognitive-emotional processing (which have mostly focused on memory processes, see e.g., Lupien et al., 2007, for a review), our results showed effects of cortisol administration on very early attentional processing of negative emotional stimuli. It would be valuable to link these two areas of research and to implement measurement of ERPs in memory studies, to investigate whether the effects of cortisol on memory are possibly related to modulation of early processing of emotional information.

Clinical implications

First, it is important to note that the approach of this study was fundamental: We aimed to gain more insight in basic processes underlying fear and avoidance in social anxiety. As a result, our findings cannot be readily used for the treatment of this disorder. Nevertheless, some of our findings do have implications for clinical practice. For example, the demonstrated link between high stress-induced cortisol levels and increased social avoidance behavior in patients with SAD (*Chapter 3*) suggests that in order to achieve optimal benefits, i.e., to decrease avoidance and promote threat approach behavior, the level of stress during exposure therapy should be kept to a limit. In addition, individual glucocorticoid stress-sensitivity might be assessed to tailor and fine tune psychological and pharmacological interventions in patients with SAD.

Also the results of our cortisol administration studies are relevant for clinical practice, as glucocorticoid administration has been proposed as a treatment for anxiety disorders (SAD and PTSD; see e.g., De Quervain, 2008; Soravia et al., 2006). This practice is based on findings showing that pre-treatment with cortisol decreases subjective anxiety during exposure to social stress, which is suggested to be mediated by glucocorticoid inhibition of fear memory (Soravia et al., 2006). Although our results do not necessarily contradict these findings, they do show additional effects of cortisol on very early attention to threat, and suggest that the direction of these effects are sensitive to the task context (*Chapters 4* and *5*), and perhaps also the conditions under which cortisol is administered (e.g., with or without concurrent social stress, see *Chapter 4* for a more extensive discussion). This context-sensitivity, along with the finding that cortisol increased early threat processing during avoidance in high anxious individuals, also argues against a general anxiolytic (fear-reducing) effect of cortisol that as has been

proposed by some researchers (e.g., Putman et al., 2007b). Thus, we propose that more research is warranted to investigate the effects of cortisol administration on processes other than memory (and subjective anxiety), and that attention should be paid to task-factors and the situation in which cortisol is administered.

Finally, it is worthwhile to investigate whether avoidance tendencies can be trained away. The results of several recent studies (e.g., Amir, Weber, Beard, Bomyea, & Taylor, 2008; MacLeod, Rutherford, Campbell, Ebsworthy, & Holker, 2002; See, Macleod, & Bridle, 2009) suggest that individuals can be trained to direct their attention away from threatening stimuli in computer tasks, and that this training reduces trait anxiety scores and anxiety responses to a subsequent environmental stressor. These findings provide support for the notion that such attentional biases play a causal role in the etiology and maintenance of anxiety. It is an interesting question to investigate whether the same is true for behavioral avoidance tendencies.

To conclude

The aim of this thesis was to gain more insight in the psychobiological mechanisms underlying social fear and avoidance behavior in humans. Our findings provide support for the important role of early threat processing and avoidance behavior in social anxiety. The findings further confirmed our prediction that cortisol plays an important role in the regulation of social avoidance behavior. This prediction was largely based on animal research, and the studies presented in this thesis are among the first to provide evidence supporting such a role of cortisol in humans. Furthermore, the results of our studies suggest that the effects of cortisol on early threat processing (and avoidance) are influenced by factors related to the motivational context, such as motivational significance and personal relevance of the stimulus material, and the task-goal.

More research investigating the effects of cortisol on early attentional processes is warranted, to study how these are related to subsequent cognitive processes, behavioral responses and subjective anxiety, and to systematically investigate the context-dependency of these effects.

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Samenvatting

Toenaderen of vermijden. Neurobiologische mechanismen in sociale angst

Het doel van dit proefschrift was om meer inzicht te krijgen in de psychobiologische mechanismen die een rol spelen bij sociale angst en vermijdingsgedrag bij mensen. Sociale angst (of sociale fobie) is de meest voorkomende angststoornis en wordt gekenmerkt door een sterke angst om negatief beoordeeld te worden door anderen, en door vermijding van sociale situaties. Experimenteel onderzoek naar sociale angst heeft zich grotendeels gericht op de rol van afwijkingen in de verwerking van sociaal-emotionele informatie, zoals aan de ene kant verhoogde aandacht (vigilantie) voor sociaal dreigende informatie en aan de andere kant vermijding van de verdere verwerking van deze informatie. Hoewel in dit proefschrift ook aandacht besteed wordt aan (met name de temporele aspecten van) deze informatieverwerking, staat het *gedrag* (toenadering of vermijding) in reactie op sociaal-emotionele informatie centraal. Experimenteel onderzoek waarin dit gedrag systematisch wordt onderzocht was namelijk schaars, hoewel de neiging tot vermijding een belangrijk kenmerk is van de sociale angststoornis en een rol kan spelen bij het in stand houden van de angst. Een tweede hoofdlijn in dit proefschrift richt zich op de rol van cortisol, een belangrijk stresshormoon, bij de regulatie van sociaal (angst-)gedrag. Uit dieronderzoek en onderzoek bij baby's is gebleken dat er een verband bestaat tussen verhoogde niveaus van glucocorticoïden (GC, cortisol bij mensen) en angstig en vermijdend gedrag in sociale situaties. Bovendien hebben deze GC een sterke invloed op prefrontale hersengebieden die ook een belangrijke rol spelen bij de regulatie van sociaal gedrag. Deze resultaten leiden tot de hypothese dat cortisol een belangrijke rol speelt bij de (prefrontale) regulatie van sociaal angstgedrag. In dit proefschrift is getracht om meer inzicht te krijgen in de verwerking van sociaal dreigende informatie en vermijdingsgedrag bij sociale angst en hoe deze processen beïnvloed worden door cortisol. Daarbij werden de volgende voorspellingen getoetst:

1. Sociaal dreigende informatie wordt door sociaal angstige individuen met verhoogde aandacht (vigilantie) verwerkt en deze vigilantie vindt plaats in vroege fasen van de informatieverwerking.

2. Mensen die gekenmerkt worden door een hoge neiging tot vermijding (*behavioral inhibition*) of sociale angst laten sterkere vermijdingsresponsen zien in reactie op sociaal dreigende informatie in vergelijking met laag vermijdende of niet sociaal-angstige individuen.
3. De verwerking van sociaal dreigende informatie en de neiging tot vermijding worden bevorderd door hoge endogene of exogene cortisolniveaus.

In *Hoofdstuk 2*, *3* en *4* werd het toenaderings- en vermijdingsgedrag in reactie op positieve en dreigende sociale stimuli onderzocht met behulp van een reactietijd computertaak (de approach–avoidance taak, Rotteveel & Phaf, 2004), waarin deelnemers de emotionele uitdrukking van foto's van boze en blije gezichten evalueerden door het maken van een toenaderende (arm flexie) of vermijdende (arm extensie) beweging. De taak bestond uit een affect-congruente conditie met toenadering van blije en vermijding van boze gezichten en een affect-incongruente conditie met toenadering van boze en vermijding van blije gezichten. Kortere reactietijden in de congruente ten opzichte van de incongruente conditie (het congruentie-effect) reflecteren de relatieve neiging om sociaal positieve stimuli toe te naderen en sociale dreiging te vermijden. Naast het gedrag werd ook de verwerking van de sociaal-emotionele informatie tijdens de taak nader onderzocht (*Hoofdstuk Twee* en *Vier)* door het meten van stimulusgerelateerde hersenactiviteit (*event-related potentials*, ERPs) met behulp van elektroden op de schedel. Met dezelfde methode werd in *Hoofdstuk 5* tijdens een andere taak, de emotionele Stroop taak, onderzocht of de verhoogde aandacht voor sociaal dreigende informatie (boze gezichten) bij patiënten ook optreedt wanneer deze informatie irrelevant is voor het doel van de taak (kleurbenoemen) en zelfs buiten de bewuste waarneming (subliminaal) wordt gepresenteerd.

De effecten van cortisol en individuele verschillen in de neiging tot vermijding/ sociale angst op de informatieverwerking en gedrag werden onderzocht door middel van orale toediening van cortisol en placebo bij hoog en laag vermijdende/angstige studenten (*Hoofdstuk 2* en *7*) en patiënten met een sociale angststoornis (*Hoofdstuk 4* en *5*), en door manipulatie van endogene (natuurlijke) cortisol niveaus met behulp van een sociale stress taak (de Trier Social Stress Test) bij patiënten met een sociale angststoornis in vergelijking met patienten met een posttraumatische stress stoornis

(PTSS) en gezonde vrijwilligers (*Hoofdstuk 3*). Hieronder volgt een kort overzicht van de belangrijkste bevindingen.

De voorspelling dat sociaal dreigende informatie door sociaal angstige individuen met verhoogde aandacht wordt verwerkt en dat deze vigilantie plaatsvindt in vroege fasen van de informatieverwerking werd duidelijk ondersteund door de bevindingen in *Hoofdstuk 5*. In deze studie vonden we bij patiënten met sociale angst verhoogde vroege ERP amplitudes (~150-250 ms na stimulusaanbieding) in reactie op boze gezichten tijdens de emotionele Stroop taak, zelfs bij beperkt bewustzijn van de stimuli en terwijl verwerking van de emotionele informatie niet nodig was voor uitvoering van de taak. Het feit dat deze vigilantie gereflecteerd werd in de ERPs maar niet in de gedragsresultaten benadrukt de meerwaarde van het gebruik van deze psychofysiologische maat. De ERP resultaten tijdens de approach-avoidance taak in twee andere studies duidden ook op een versterkte verwerking van sociaal dreigende stimuli bij hoog vermijdende studenten (*Hoofdstuk 2*) en patiënten met ernstige sociale angstklachten (*Hoofdstuk 4*), maar alleen tijdens het uitvoeren van vermijdende reacties en na cortisol toediening.

De tweede voorspelling was dat mensen die gekenmerkt worden door een hoge neiging tot vermijding (*behavioral inhibition*, gemeten met een zelfrapportage vragenlijst) of sociale angst, een sterkere neiging tot vermijding laten zien in reactie op sociaal dreigende informatie dan laag vermijdende of niet-sociaal angstige individuen. Deze voorspelling werd gedeeltelijk ondersteund door de bevindingen in *Hoofdstuk 4*, waarin patiënten met ernstiger angstklachten een vertraging in de toenaderingsreacties op boze gezichten lieten zien, in overeenstemming met een sterkere neiging deze stimuli te vermijden. Onder basale omstandigheden (dwz. na placebo toediening of voor stress inductie) vonden we echter geen significant sterkere neiging tot vermijding van boze gezichten bij hoog versus laag vermijdende studenten (*Hoofdstuk 2*) of bij patiënten met een sociale angststoornis in vergelijking met patiënten met PTSS of mensen zonder angstklachten (*Hoofdstuk 3*). We vonden wel ondersteuning voor verhoogde neigingen tot vermijding van sociale dreiging in hoog angstige individuen in de context van verhoogde cortisol niveaus (zie hieronder).

De derde belangrijke voorspelling in dit proefschrift was dat verhoogde cortisolniveaus (door toediening of sociale stress) de verwerking en vermijding van

sociaal dreigende informatie zouden bevorderen. Deze voorspelling werd grotendeels ondersteund door de bevindingen in de *Hoofdstukken 2, 3* en *4*. In de studie in *Hoofdstuk 2* resulteerde cortisol-toediening bij hoog vermijdende studenten op de approach-avoidance taak in een toename van het congruentie-effect voor boze gezichten, wat duidt op een relatieve toename in de neiging om deze stimuli te vermijden. Daarnaast bleek in *Hoofdstuk 3* dat sociale stress bij patiënten met een sociale angststoornis, in vergelijking met patiënten met PTSS en gezonde vrijwilligers, leidde tot sterkere cortisolstijgingen en een toename in vermijdingsreacties op boze gezichten. De bevinding dat deze toename in vermijdingsgedrag significant samenhing met de verhoogde cortisol reacties, zelfs wanneer gecontroleerd werd voor andere stress-gerelateerde effecten zoals verhoogde bloeddruk of subjectieve angst, duidt op een directe relatie tussen verhoogde cortisol stress-responsiviteit en sociaal vermijdingsgedrag bij patiënten met een sociale angststoornis. Naast deze gedragseffecten lieten de ERP resultaten van de approach-avoidance taak, in overeenkomst met de voorspelling, na cortisol toediening ook een facilitatie zien in de verwerking van sociaal dreigende stimuli bij hoog vermijdende studenten (*Hoofdstuk 2*) en patiënten met een sociale angststoornis (*Hoofdstuk 4*). Opvallend was dat deze facilitatie alleen optrad tijdens de uitvoering van vermijdingsreacties, wat er op kan duiden dat de relatieve facilitatie van het vermijdingsgedrag tot stand komt via een toename in de vroege aandacht voor dreiging. In tegenstelling tot de resultaten van *Hoofdstuk 2* en *4*, leidde cortisol toediening in *Hoofdstuk 5* tot een significante *afname* in de vroege verwerking van sociaal dreigende informatie tijdens gemaskeerde (onderbewuste) stimuluspresentatie. Deze schijnbare tegenstelling kan verklaard worden door het feit dat de evaluatie van de boze gezichten in *Hoofdstuk 5* irrelevant was en interfereerde met het taakdoel (kleurbenoemen), terwijl in de approach-avoidance taak (*Hoofdstuk 2* en *4*) de emotionele evaluatie van de stimuli expliciet was en van belang voor de uitvoering van doelgericht sociaal gedrag. Samen duiden deze bevindingen erop dat cortisol de verwerking van sociaal dreigende informatie op een taak-relevante manier beïnvloedt. Dit is in overeenstemming met de suggestie, afkomstig uit dieronderzoek, dat GC effecten op cognitie over het algemeen adaptief zijn en de verwerking van informatie en het gedrag faciliteren welke het meest relevant zijn voor de betreffende situatie (De Kloet, Oitzl, & Joëls,1999).

In *Hoofdstuk 6* stond een meer theoretisch-methodologische vraag centraal, namelijk wat het effect is op de evaluatie van boze en blije gezichten als de stimuli in plaats van de deelnemers een toenaderende of vermijdende beweging maken. Uit de resultaten van een serie reactietijd experimenten bleek dat, vergelijkbaar met het congruentie-effect op de approach-avoidance taak, deelnemers sneller reageerden en minder fouten maakten in reactie op toenaderende blije en terugtrekkende/vermijdende boze gezichten dan bij toenaderende boze en terugtrekkende blije gezichten. Deze resultaten laten zien dat dergelijke congruentie-effecten niet afhankelijk zijn van een actieve beweging van de deelnemer en duiden erop dat de relatieve afstand tussen de deelnemer en de stimulus een belangrijke rol speelt bij deze effecten.

In *Hoofdstuk 7*, ten slotte, werd het effect van cortisol toediening vs. placebo onderzocht op de hersenactiviteit in rust (gemeten met behulp van EEG elektroden op de schedel) bij hoog en laag vermijdende/angstige studenten (dezelfde als in *Hoofdstuk 2*). Bij de hoog vermijdende, maar niet bij de laag vermijdende studenten vonden we in beide condities een patroon van hersenactiviteit dat in eerder onderzoek in verband is gebracht met zowel angstige en vermijdende gemoedstoestanden als verhoogde basale endogene cortisol niveaus. Dit patroon, een significante correlatie tussen delta en beta EEG activiteit op mid-frontale elektrodes, reflecteert mogelijk een toename in de informatiestroom tussen corticale en subcorticale hersengebieden. Cortisol toediening leidde, in vergelijking met placebo, met name bij de hoog vermijdende studenten tot een verdere toename van deze correlatie. De causale invloed van cortisol op deze toestand van de hersenen kan belangrijke gevolgen hebben voor de informatieverwerking en gedrag.

De bevindingen in dit proefschrift bieden niet alleen experimentele ondersteuning voor de belangrijke rol van verhoogde vroege aandacht voor en vermijding van sociaal dreigende informatie in sociale angst, maar geven ook inzicht in de condities waaronder deze neigingen het sterkst optreden. Bovendien onderschrijven de resultaten de invloed van cortisol op de regulatie van deze processen en tonen ze aan dat deze invloed context-afhankelijk is. Dat wil zeggen dat de sterkte en de richting van het effect van cortisol afhangen van factoren zoals de fase van de informatieverwerking, het motivationele belang of de persoonlijke relevantie van de informatie en het taakdoel. Hierbij blijkt het effect van cortisol het sterkst op de vroege verwerking van

motivationeel belangrijke informatie (signalen van sociale dreiging) bij personen die bijzonder gevoelig zijn voor deze informatie (hoog vermijdende of sociaal angstige individuen) en lijkt het de informatieverwerking en het gedrag te faciliteren dat past bij het taakdoel.

Hoewel het onderzoek in dit proefschrift fundamenteel was en gericht op het verkrijgen van meer inzicht in basale processen die een rol spelen bij angst en vermijdingsgedrag in sociale angst, zijn de resultaten ook relevant voor de klinische praktijk. Zo wijst de gevonden relatie tussen verhoogde cortisol stress-responsiviteit en sociaal vermijdingsgedrag bij patiënten met een sociale angststoornis (*Hoofdstuk 3*) op het belang om het stressniveau tijdens "exposure" therapie te beperken, teneinde vermijdingsgedrag te verminderen en toenadering te bevorderen. Daarnaast zijn onze resultaten relevant voor de voorgestelde toepassing van cortisol toediening bij behandeling voor angststoornissen zoals sociale angst en PTSS (e.g., De Quervain, 2008; Soravia et al., 2006). Dit voorstel is gebaseerd op een afname in subjectieve angst tijdens blootstelling aan sociale stress na toediening van cortisol, mogelijk als gevolg van effecten op het geheugen. Onze bevindingen waaruit blijkt dat cortisol toediening van invloed is op zeer vroege verwerking van sociaal-emotionele informatie pleiten voor meer aandacht voor het effect van cortisol op andere processen dan subjectieve angst (of geheugen). De bevinding dat de richting van dit effect gevoelig is voor de taak-context benadrukt bovendien het belang van aandacht voor de situatie waarin cortisol wordt toegediend.

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Curriculum Vitae

Jacobien Marit van Peer was born on February 17, 1979 in Zuidhorn, the Netherlands. She graduated from high school at the Praedinius Gymnasium in Groningen in 1997, after which she began studying psychology at the Rijksuniversiteit in Groningen. She specialized in experimental and neuro-/biological psychology, and obtained her *Doctoraal* / Master of Science degree (cum laude) in November 2002 after a research internship at the department of Biological Psychiatry of the Groningen University Medical Center. This research, supervised by Dr. Mattie Tops, concentrated on the acute effects of cortisol on frontal EEG alpha asymmetry, emotional memory, and mood in patients with a major depressive disorder. In February 2004 she started her PhD research at the department of Clinical, Health and Neuropsychology of Leiden University, which forms the basis of this dissertation. The studies in this project focused on the acute effects of cortisol on social threat processing and approach and avoidance behavior in social anxiety, and were supervised by Prof. dr. Philip Spinhoven and Dr. Karin Roelofs. In November 2009 she began working as a post-doctoral researcher at the Swiss National Center for Competence in Research (NCCR) in Affective Sciences/ Centre Interfacultaire en Sciences Affectives (CISA) of the University of Geneva with Prof. dr. Klaus Scherer as part of an extensive research project on the production and perception of emotion.

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