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Psychogenic non epileptic seizures : towards an integration of psychogenic, cognitive and neurobiological aspects

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PSYCHOGENIC NON EPILEPTIC SEIZURES

towards an integration of psychogenic, cognitive and neurobiological aspects

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CHAPTER 1

General Introduction

Psychogenic Non Epileptic Seizures (PNES) are defined as paroxysmal involuntary behavioral patterns that mimic epileptic events –covering the full range of hypo-motor events to hyper-motor manifestations– but that lack ictal epileptiform activity in the brain. PNES cannot be fully explained by any known neurological or other somatic diseases and are thought to be mediated by psychological factors (WHO, 1993; APA, 1994). PNES are characterized by a sudden and time-limited alteration of consciousness and are associated with a disturbance in controlling cognitive, behavioral and/or emotional functions (Kuyk et al., 1999).

Epidemiology

Because their symptoms have a neurological appearance but a psychogenic origin, patients with PNES find themselves on the verge of the medical and mental health services, although most patients are seen in tertiary epilepsy centers. The incidence of PNES in the general population has been estimated as 1.5 to 33 per 100.000 persons per year (see Reuber, 2008). In 25-30% of the patients referred to tertiary epilepsy centers for refractory epilepsy a diagnosis of PNES is obtained, of whom 5 to 40% have a (history of) concomitant epilepsy diagnosis (for reviews see e.g. Reuber, 2008; Bodde et al., 2009). Besides the high comorbidity rate with epilepsy, PNES is also associated with high rates of psychiatric comorbidity, especially anxiety and depressive symptoms (for a review see e.g. Bodde et al., 2009). The female-male ratio is approximately 3:4 (Alper, 1994; Lesser, 1996) and PNES typically starts in the second or third decade of life, although seizure onset below age 4 and above 70 has also been described (see Reuber, 2008).

Diagnosis

In most patients, there is a delay of several years between the manifestation of PNES and the correct diagnosis (De Timary et al., 2002; Reuber et al., 2002, Kuyk et al., 2008) which has alarming consequences. For instance most PNES patients initially receive antiepileptic drugs (AEDs- De Timary et al., 2002), while these have been observed to be ineffective or may even worsen PNES (see LaFrance & Devinsky, 2002). Furthermore, besides the personal costs that are associated with an incorrect neurological diagnosis for patients and their families, it has been estimated that misdiagnosis and mistreatment of PNES as epilepsy cost the US health services 110-920

million dollars annually on repetitive laboratory studies, diagnostic evaluations, inappropriate AEDs and emergency department utilization (Martin et al., 1998; LaFrance & Benbadis, 2006).

The gold standard for PNES diagnosis is an ictal video-EEG registration of a typical seizure to confirm the absence of epileptiform activity during a seizure (see e.g. LaFrance, 2008). Admission to an epilepsy monitoring unit (EMU) has been described to provide a definitive diagnosis in almost 90% of patients, and rectifies an incorrect diagnosis of epilepsy in a considerable proportion of patients (see LaFrance, 2008).

Classification

The term PNES is a neurological idiom that will be used consistently in the present thesis. In the psychiatric manuals however, PNES are classified as one of the major manifestations of conversion disorder as described in the *DSM-IV* (APA, 1994). In *ICD-10* (WHO, 1993) PNES are categorized under dissociative disorders, more specifically under dissociative convulsions. Importantly, both classification systems specify that the symptoms cannot be fully explained by a medical condition and that the etiology of PNES is related to psychological stress factors (see Table 1.1 and Table 1.2 for an overview of the complete diagnostic criteria for PNES defined by *DSM-IV* and *ICD-10* respectively).

Table 1.1. Overview of diagnostic criteria for PNES as stated by the DSM-IV.

DSM – IV (APA, 1994): Conversion disorder – subtype with seizures or convulsions
A. One or more symptoms or deficits affecting voluntary motor or sensory function that suggest a neurological or other general medical condition. B. Psychological factors are judged to be associated with the symptom or deficit because the initiation or exacerbation of the symptom or deficit is preceded by conflicts or other stressors. C. The symptom or deficit is not intentionally produced or feigned (as in Factitious Disorder or Malingering). D. The symptom or deficit cannot, after appropriate investigation, be fully explained by a general medical condition, or by the direct effects of a substance, or as a culturally sanctioned behavior or experience. E. The symptom or deficit causes clinically significant distress or impairment in social, occupational, or other important areas of functioning or warrants medical evaluation. F. The symptom or deficit is not limited to pain or sexual dysfunction, does not occur exclusively during the course of Somatization Disorder, and is not better accounted for by another mental disorder.

Table 1.2. Description of PNES as stated by the ICD-10.

ICD-10 (WHO, 1993): Dissociative [conversion] disorders - subtype: dissociative convulsions
The common themes that are shared by dissociative or conversion disorders are a partial or complete loss of the normal integration between memories of the past, awareness of identity and immediate sensations, and control of bodily movements. All types of dissociative disorders tend to remit after a few weeks or months, particularly if their onset is associated with a traumatic life event. More chronic disorders, particularly paralyses and anaesthesias, may develop if the onset is associated with insoluble problems or interpersonal difficulties. These disorders have previously been classified as various types of "conversion hysteria". They are presumed to be psychogenic in origin, being associated closely in time with traumatic events, insoluble and intolerable problems, or disturbed relationships. The symptoms often represent the patient's concept of how a physical illness would be manifest. Medical examination and investigation do not reveal the presence of any known physical or neurological disorder. In addition, there is evidence that the loss of function is an expression of emotional conflicts or needs. The symptoms may develop in close relationship to psychological stress, and often appear suddenly. Only disorders of physical functions normally under voluntary control and loss of sensations are included here. Disorders involving pain and other complex physical sensations mediated by the autonomic nervous system are classified under somatization disorder. The possibility of the later appearance of serious physical or psychiatric disorders should always be kept in mind. Includes: conversion: · hysteria, · reaction, hysteria, hysterical psychosis. Excludes: malingering [conscious simulation].

Psychological stress factors

Support for the assumption that PNES are associated with psychological stress factors has been found in self-report studies describing that patients with PNES commonly report increased rates of (childhood) psychological trauma compared to both neurological and healthy control groups (for reviews see Fiszman et al., 2004; Sharpe & Faye, 2006; Roelofs & Spinhoven, 2007). Further findings from self-report investigations indicated that patients with PNES experience their lives as more stressful and use more maladaptive avoidant coping strategies, i.e. behavioral efforts to avoid threatening or stressful situations (Frances et al., 1999; Goldstein et al., 2000; 2006). Recently, in his biopsychosocial model (2009), Reuber formulated childhood abuse and neglect as predisposing factors, that is an increased vulnerability, to develop PNES in later life (see also Bodde et al, 2009). Based on one study in which patients with recent-onset PNES reported more negative life events in the year prior to seizure onset compared to patients with recent-onset epilepsy (Binzer et al, 2004), Reuber (2009) further suggested that negative life events in adulthood, temporally preceding

the onset of the seizures, could be considered as a precipitating factor, meaning that these adult life events seemed to cause the PNES to start. Evidence for such process in a large group of patients with mixed conversion complaints, including PNES, was found by Roelofs et al. (2005b), showing that the relation between early trauma and later conversion symptoms was partially mediated by recent negative life-events (Roelofs et al., 2005b). According to Reuber, patients' subsequent avoidance behavior to deal with life stressors forms another important precipitating and perpetuating factor in PNES, making patients unable to regain control of their seizures or even aggravating the seizures (Reuber, 2009; see also Bodde et al., 2009). Although Reuber acknowledges (early) psychological trauma and stress as an important etiological factor for the development of PNES, and subsequent avoidant behavior in response to threat and stress as an important factor maintaining the disorder, his descriptive model does not provide an explanation of *how* these factors may result in the paroxysmal disintegration of important cognitive and behavioral functions associated with PNES.

Underlying mechanisms

Janet (1907) and later also the (neo-) dissociation theorists (Hilgard, 1977; Kihlstrom, 1992; Brown, 2004) have theorized on possible mechanisms underlying conversion and dissociative phenomena such as PNES. They regarded PNES as attention-related complaints due to psychological stress factors. Janet for example proposed, based on observational studies, that these symptoms result from an impairment of the attentional functions due to severe stress or trauma. Although these (neo-) dissociation theories are still influential in recent theoretical models and therapeutic interventions with respect to dissociative and conversion symptoms, Roelofs and Spinhoven (2007) recently argued that these cognitive models lack empirical evidence and should integrate recent findings of neurobiological stress research.

Contemporary neurobiological stress research

Below we will describe a general stress model that has been implicated in a wide range of psychiatric disorders including conversion disorder (McEwen, 1998, see Roelofs and Spinhoven, 2007 p. 812-3). "An individuals' response to stress is generated by a network of integrative brain structures involving subregions of the hypothalamus, amygdala and periaqueductal gray. These structures receive input from visceral and somatic afferents and from cortical structures, in particular the ventral subdivision of the anterior cingulated cortex (ACC) and medial prefrontal cortices. This integrative network provides outputs to the pituitary and to the pontomedullary nuclei. The latter structures respectively mediate the neuroendocrine and autonomic output of the body. This central stress circuitry is under feedback control via noradrenergic and serotonergic projections from the brainstem and via glucocorticoid pathways, which exert an inhibitory control via glucocorticoid receptors located in the hippocampus and the medial prefrontal cortex. The stress-response of this central circuitry includes responses of the Hypothalamus Pituitary Adrenal (HPA)-axis and the autonomic nervous system. The individuals' stress-responsiveness is not only under genetic control but is also influenced by early traumatization and forms of pathological stress, which may result in long lasting and even permanent changes in the central stress circuitry" (e.g. Sapolsky, 1997; Anisman et al., 1998; Heim et al., 2001; Elzinga et al., 2003; see Roelofs and Spinhoven, 2007). The deregulatory effect of stress and trauma on the HPA-axis with its end-product cortisol has gained great attention. This is of particular interest to PNES since early trauma has been described as a predisposing factor in the development of PNES (Reuber, 2009), which makes patients' central stress system more vulnerable to the effects of later stressors, that in turn serves as a precipitating factor for PNES onset (Reuber, 2009, see also Roelofs & Spinhoven, 2007). Secondly, recent findings linking increased cortisol to important cognitive integrative impairments (e.g. Lupien et al., 1999; Elzinga & Roelofs, 2005; Oei et al., 2009) suggest a pathway how stress may result in the paroxysmal impairment of cognitive functions characteristic for PNES. Third, the recently reported positive association between cortisol and threat avoidance behavior (Roelofs et al., 2005a; 2009a; Van Peer et al., 2007; 2009) may provide a model for the increased tendency to avoid threat or stressful situations in patients with PNES (e.g. Reuber, 2009).

To summarize: PNES are considered as a paroxysmal disintegration of cognitive functions associated with psychological stress factors. Self-report studies have found indications of increased stress sensitivity in patients with PNES, and psychological stress and trauma, as well as subsequent maladaptive avoidant behavior to deal with threatening and stressful situations have been acknowledged as important etiological factors in PNES (e.g. Reuber, 2009). The primary aim of the present thesis was to use an integrative approach of cognitive and neurobiological stress research to test the assumptions of increased cognitive and neurobiological stress sensitivity in patients with PNES. Secondly, we aimed to investigate how possible findings of increased cognitive and neurobiological stress sensitivity may influence a) important cognitive integrative functions, b) avoidance behavior in patients with PNES.

In the next paragraphs, we will detail the results of previous studies investigating both cognitive and neurobiological indications for increased stress sensitivity in patients with PNES, which is followed by a brief outline of the additional value of the methodology used in the studies described in the current thesis. The overview ends with a description of the main hypotheses, and an outline of the studies described in each of the remaining chapters of this thesis.

Cognitive threat sensitivity

Although studies investigating the effects of stress on cognitive functioning in patients with PNES are scarce, standard neuropsychological test batteries have demonstrated a wide range of cognitive impairments in patients with PNES compared to healthy controls (HCs) including memory and attentional problems (for reviews see Cragar et al., 2002; Binder & Salinsky, 2007). Although recently there has been a debate whether these cognitive abnormalities in patients with PNES might be caused by poor effort during task performance (Cragar, 2006; Drane et al., 2006; Locke et al., 2006; Binder & Salinsky, 2007; Dodrill, 2008). The only study reporting the additional effect of stress-induction on cognitive performance in patients with PNES, was performed by Bendefeldt et al. (1976) who examined attentional processing in 17 patients with conversion symptoms (10 were suffering from PNES). Although they did find evidence for worsened attentional processing (compared to a non-psychotic patient

control group) both at baseline and following stress using a face recognition task and a mental switch-task, they did not check whether stress-induction resulted in an actual (neuro)biological stress-response. Moreover, studies so far only investigated the cognitive processing of neutral stimuli, no studies have reported the effects of relevant stress cues on the cognitive processing in patients with PNES (Ludwig, 1972).

In addition to the previous reported neuropsychological studies in patients with PNES, we investigated the cognitive threat sensitivity in patients with PNES by testing the cognitive processing of relevant threat stimuli. Angry facial expressions have been found to be important threat cues in cognitive processing. Several neuroimaging studies have shown that viewing angry faces activates limbic structures, the amygdala in particular (for an overview see Adolphs et al., 2002; McClure et al., 2004; Strauss, et al., 2005b), supporting the relevance of these stimuli in the study of stress related disorders and the role of interpersonal trauma, in particular. We therefore expected these social threat cues to be of relevance to patients with PNES, particularly for those patients reporting a history of *interpersonal* psychological trauma. Secondly, we tested patients' cognitive threat sensitivity by testing the cognitive processing of both neutral and threat stimuli at baseline and in a stress-context, using stress-induction protocols. To check if stress-induction was successful, several physiological stress parameters, e.g. cortisol, were assessed throughout the experiment. Possible findings were furthermore linked to cortisol and psychological trauma reports.

Moreover, in addition to the increased avoidance coping in response to threat and stress commonly reported by patients with PNES (Frances et al., 1999; Goldstein et al., 2000; 2006; Reuber, 2009), we tested actual avoidance *behavior* in response to angry facial expressions in patients with PNES. Therefore, in addition to the cognitive processing of threat stimuli, threat avoidance behavior in patients with PNES was also assessed at baseline and in a stress-context. Furthermore, these behavioral threat avoidance tendencies were linked to cortisol.

Neurobiological stress sensitivity

Only few studies have investigated the association of PNES with the HPA-axis stress system with cortisol as its end-product. The majority of these studies focused on the effects of seizure-like activity on cortisol levels and mostly found increased cortisol levels in patients with PNES

(as well as in confirmed epilepsy patients) related to seizures (e.g. Mehta et al., 1994; Tunca et al., 2000). So far, only two studies have investigated *basal* activity of the HPA-axis in PNES and the results are conflicting. Tunca et al. (1996) did not find increased basal cortisol levels in a sample of 25 patients with conversion disorder (including 20 PNES patients) compared to HCs but did find decreased cortisol suppression after dexamethasone administration. In contrast, in a sample of eight PNES patients, Tunca et al. later (2000) observed increased morning serum cortisol levels at baseline (an average time interval of 18 hours had elapsed since the last seizure). These conflicting findings may be caused by the fact that only a few time-points were measured to establish HPA-axis activity and may further be due to a lack of control for relevant factors such as comorbid psychopathology, use of psychotropic medication and smoking behavior.

Based on the conflicting results of Tunca and colleagues (1996; 2000) we tested several relevant aspects of the HPA-axis in patients with PNES by collecting cortisol saliva samples on 19 time-points on two consecutive days. Importantly, besides the extensive sampling schedule, relevant demographic and patient characteristics were matched or statistically controlled for.

Main hypotheses

In the present thesis, the following hypotheses have been tested: 1). Patients with PNES display increased cognitive threat sensitivity. 2). Patients with PNES display increased neurobiological stress sensitivity. 3). Patients' increased cognitive and neurobiological stress sensitivity a) interfere with crucial cognitive integrative functions and b) are positively associated with increased threat avoidance behavior.

In total, two experimental laboratory studies have been conducted in which several cognitive functions as well as threat behavior were assessed at baseline and following two different stress-induction procedures. A third study was performed to test several HPA-axis functions on two consecutive stress-free days.

In all three studies the experimental group consisted only of PNES patients who had been diagnosed based on the gold standard, that is an ictal video-EEG registration. The control group consisted of matched healthy control participants without a psychiatric or medical diagnosis.

We furthermore aimed to include a second control group consisting of patients with epilepsy, but due to the complexity of relevant factors that had to be taken into account (type of epilepsy, polytherapy AED, age and gender differences and excessive smoking to name a few) and because the long neuropsychological testing sometimes produced epileptic seizures, we were able to include their results only marginally in Chapter 3 (see below).

Overview of chapters

Chapter 2. In this laboratory experiment we examined the first hypothesis of increased cognitive threat interference in patients with PNES by investigating the attentional processing of social threat cues in patients with PNES in relation to interpersonal trauma and acute psychological stress. Therefore, a masked emotional Stroop test, comparing color-naming latencies for backwardly masked angry, neutral and happy faces, was administered to 19 unmedicated patients with PNES and 20 matched HCs, at baseline and in a stress condition. Stress was induced by means of the Trier Social Stress Test (TSST- a public speaking task) and physiological stress parameters, such as heart rate variability (HRV) and cortisol, were measured throughout the experiment. We expected patients with PNES, particularly patients reporting interpersonal psychological trauma, to show a positive attentional bias for angry faces, which would be most pronounced in the stress-context.

Chapter 3. In this chapter we investigated whether patients with PNES displayed increased neurobiological stress sensitivity by testing several relevant HPA-axis functions in PNES patients and related them to trauma history. Cortisol awakening curve, basal diurnal cortisol and negative cortisol feedback (using a 1 mg Dexamethasone-Suppression-Test) were examined in 18 PNES patients and 19 matched HCs using saliva cortisol sampling on two consecutive days at 19 time-points. Concomitant sympathetic nervous system (SNS) activity was assessed by analyzing saliva alpha-amylase (sAA). We expected to find increased cortisol levels in the patients group, especially in patients reporting psychological trauma.

Chapter 4. This chapter provides the first integration of cognitive and neurobiological findings in patients with PNES. We reanalyzed the previously described emotional Stroop data (Chapter 2) and related the previously reported attentional processing of angry faces to newly

analyzed baseline (pre-task) cortisol levels in the 19 unmedicated patients with PNES and the 20 HCs. In addition, we tested the specificity of eventual effects by investigating the same relationship in a new control group of 17 patients with epileptic seizures. We expected that only in patients with PNES pre-task cortisol levels would be positively associated with the increased interference of the attentional processing of angry faces.

Chapter 5. In this chapter we tested the first part of the third hypothesis, that is whether the increased cognitive threat and neurobiological stress sensitivity in patients with PNES interfered with crucial integrative cognitive functions. An important cognitive function needed for almost every voluntary action is working memory (WM). WM performance in 19 patients with PNES and matched HCs was tested by administrating a N-back task with emotional distracters (photos of angry, happy and neutral faces), requiring participants to monitor sequences of letters in various cognitive loads and to ignore the distracters, at baseline and after stress-induction (Cold Pressor Test). Saliva cortisol was measured throughout the experiment. We expected to find increased WM interference by angry face distracters in patients with PNES already at baseline, followed by a generalization of WM impairment by the social distracters following stress-induction, which we expected to be positively related to stress-induced cortisol.

Chapter 6. In the same experiment as described in Chapter 5, we tested the second part of the third hypothesis of automatic threat avoidance behavioral tendencies in patients with PNES in relation to stress and cortisol levels. Due to technical problems, the approach-avoidance (AA) task data was only available for 12 patients with PNES and 20 matched HCs. The AA task requires participants to evaluate the emotional valence of pictures of angry and happy faces by making arm movements (arm flexion or extension) that are either affect-congruent (avoid-angry; approach-happy) or affect-incongruent (approach-angry; avoid-happy) with intuitive action tendencies. The AA task was administered at baseline and following stress-induction using the Cold Pressor Test (CPT) and saliva cortisol was measured throughout the experiment. We expected patients to respond faster when avoiding threat stimuli. We expected this effect to be even more pronounced following stress-induction and to be positively associated with cortisol.

Finally*, Chapter 7* provides an overview and integration of the findings of the chapters 2-6, and a discussion of the strengths and

limitations of the studies presented in this thesis. This chapter concludes with suggestions for future research and implications for clinical practice.

CHAPTER 2

Trauma, stress and preconscious threat processing in patients with Psychogenic Non Epileptic Seizures

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Abstract

Purpose. Psychogenic Non Epileptic Seizures (PNES) have long been considered as paroxysmal dissociative symptoms characterized by an alteration of attentional functions caused by severe stress or trauma. Although interpersonal trauma is common in PNES, the proposed relation between trauma and attentional functions remains under explored. We examined the attentional processing of social threat in PNES in relation to interpersonal trauma and acute psychological stress.

Methods. A masked emotional Stroop test, comparing color-naming latencies for backwardly masked angry, neutral and happy faces, was administered to 19 unmedicated patients with PNES and 20 matched healthy controls, at baseline and in a stress condition. Stress was induced by means of the Trier Social Stress Test and physiological stress parameters, such as heart rate variability (HRV) and cortisol, were measured throughout the experiment.

Results. No group differences related to the acute stress-induction were found. Compared to controls, however, patients displayed a positive attentional bias for masked angry faces at baseline, which was correlated to self-reported sexual trauma. Moreover, patients showed lower HRV at baseline and during recovery.

Discussion. These findings are suggestive of a state of hypervigilance in patients with PNES. The relation with self-reported trauma, moreover, offers the first evidence linking psychological risk factors to altered information processing in PNES.

Introduction

Psychogenic Non Epileptic Seizures (PNES) can be defined as paroxysmal involuntary behavioral patterns that mimic epileptic events but for which no organic cause can be identified. PNES lack ictal epileptiform activity in the brain and are thought to be mediated by psychological factors (World Health Organization, 1993; American Psychiatric Association, 1994). They are characterized by a sudden and time-limited alteration of consciousness and are associated with a disturbance in controlling motor, sensory, autonomic, cognitive, emotional and/or behavioral functions (e.g. Kuyk et al., 1999). It is estimated that up to 30 percent of patients referred to specialized epilepsy centres experience PNES (e.g. Gumnit, 1993; Martin et al., 2002; Benbadis, 2005) and several authors emphasize the high load that PNES patients impose on health service resources (Martin et al., 1998; LaFrance & Benbadis, 2006).

PNES form one of the major manifestations of conversion disorder as described in the DSM-IV (American Psychiatric Association, 1994). In ICD-10 (World Health Organization, 1993) PNES are categorized under dissociative disorders, more specifically under dissociative convulsions. Both classification systems specify that the etiology of PNES is related to psychological stress factors.

Previous research has shown that PNES are associated with a history of psychological trauma, such as sexual and physical abuse (e.g. Betts & Boden, 1992; Bowman, 1993; Moore & Baker, 1997; Kuyk, et al., 1999; Fiszman et al., 2004; Sharpe & Faye, 2006). However, how these increased interpersonal trauma rates may be related to PNES remains under explored.

Conversion/dissociative symptoms such as PNES have long been regarded as attention-related complaints due to psychological stress factors (Janet, 1907; Ludwig, 1972; Brown, 2004). Pierre Janet (1907); for example, proposed that these symptoms result from an impairment of the attentional functions due to severe stress or trauma. There is empirical evidence for altered attentional functioning in trauma-related disorders. For example, patients with Post Traumatic Stress Disorder (PTSD) commonly allocate their attention towards trauma-related stimuli, as evidenced by studies using the emotional Stroop task (for reviews see McNally, 1996; Buckley et al., 2000). These studies

demonstrated that PTSD patients are slower in color-naming trauma-specific threat words, as compared to trauma-unrelated words indicating that attention is allocated automatically towards the threat-value of the word (Williams et al., 1996).

These findings may be relevant for our understanding of the theorized impairments of attentional functions in patients with PNES, although studies on stress and attentional functioning in patients with PNES are scarce. Compared to healthy control groups, patients with PNES show decreased attentional functioning in standard neuropsychological test batteries (for a review see Cragar et al., 2002). There is, however, only one study in which the effects of stress on cognitive functions in PNES were examined. Bendefeldt et al. (1976) investigated attentional processing in 17 patients with conversion symptoms (10 had PNES) and found some evidence for worsened attentional processing (compared to a non-psychotic patient control group) in both baseline and stress conditions, using a face recognition task and a mental switch-task. Only the processing of neutral stimuli was, however, assessed. The processing of stimuli relevant to interpersonal trauma, such as trauma-related words or threatening faces, has not been examined. In addition, no studies have addressed the relationship between interpersonal trauma and attentional deficits in PNES.

With the present study we aimed to test the proposed relationship between attentional processing of social threat stimuli and psychological stress factors in a sample of PNES patients. We were specifically interested in testing the hypothesis that patients with PNES automatically allocate their attentional resources towards social threat stimuli. To test this hypothesis, patients and matched healthy controls were administered a masked emotional Stroop task, in which pictures of angry, happy and neutral facial expressions were presented backwardly masked and participants were asked to color name the masks (Van Honk et al., 1998, 2000; Putman et al., 2004; Hermans et al., 2006; Roelofs et al., 2007). The major outcome of emotional Stroop tasks is the attentional bias score, which is calculated by subtracting the color-naming latencies for neutral faces from the latencies needed to color-name emotional faces. A positive attentional bias score (i.e. color-naming latencies for emotional faces are larger than those for neutral faces) is taken to indicate vigilance, whereas a negative attentional bias score (i.e. color-naming latencies for emotional faces are shorter than

those for neutral faces) is thought to indicate avoidance (e.g. Mathews and MacLeod, 1994; Van Honk et al.1998, 2000; Putman et al., 2004). We used a masked version of the emotional Stroop task, in which the stimulus processing remains preconscious due to the short stimulus presentation (14 ms), making it unlikely that subjects exerted strategic effort to control possible attentional bias effects (e.g. MacLeod & Hagan, 1992; Van den Hout et al. 1995; Williams et al., 1996; Putman et al., 2004). Masked Stroop tasks have yielded more consistent results (Putman et al., 2004) and are more predictive than unmasked Stroop tasks of actual coping with stressful life-events (MacLeod & Hagan, 1992). On the basis of the previous findings in trauma-related disorders we expected that patients with PNES would show a positive attentional bias for angry faces.

Secondly, we tested whether such positive attentional bias would be related to interpersonal trauma reports in patients with PNES. Finally, we tested whether acute psychological stress affects the attentional bias towards interpersonal threat cues in patients with PNES. Therefore, we administered the Stroop task in a baseline and a social stress condition. Physiological and subjective stress markers (cortisol, heart rate, blood pressure and subjective anxiety) were assessed throughout the experiment.

Methods

Participants

Patients with PNES, who were admitted to SEIN, Epilepsy Institute in the Netherlands, were recruited by their neurologists. Inclusion criteria were: (1) diagnosis of PNES based on an ictal video-EEG (electroencephalography) recording of a typical seizure; (2) PNES is characterized by complete or partial loss of consciousness (specified as an ictal diminished or loss of adequate responsiveness or post-ictal memory impairments of the ictal event); (3) the occurrence of at least two seizures in the year prior to the experiment; (4) no history of epileptic seizure; (5) no comorbid neurological disease diagnosis; (6) no current use of antidepressants, corticosteroids, lithium, beta-blockers, cimetidine or ketoconazole; and (7) no significant endocrine disorder(s). Two of the 21 patients who participated in this study were excluded post hoc from the analysis as one was found to be using antidepressant

medication, and the other experienced a PNES during testing. The remaining patients (four males, 15 females) had a mean age of 27.58 (*SD*=7.30) years. Table 2.1 shows the subjects' demographics as well as use of contraceptives, menstrual cycle, comorbid DSM-IV axis I diagnoses (assessed using the MINI: Mini-International Neuropsychiatric Interview, Sheenan et al 1998), self-reported interpersonal traumatic experiences and seizure characteristics.

The control group was recruited through advertisements in local newspapers. Inclusion criteria were: (1) no psychiatric diagnoses assessed; (2) no clinically significant medical disease; (3) no neurological disease diagnosis; and (4) not using medication. Twenty healthy controls (two males, 18 females) with a mean age of 22.10 (*SD*=4.22) years were recruited. Table 2.1 shows that patients were slightly older than controls but did not differ with respect to educational level, gender, use of contraceptives and menstruation cycle. PNES patients reported higher rates of all types of interpersonal trauma compared to the control group.

All participants were instructed to minimize physical exercise during the hour preceding the experiment and to avoid large meals, coffee, drinks with low pH or cigarettes, because these variables can affect cortisol levels. All participants had normal or correct-to-normal vision. The study was approved by the local ethics committee and all participants provided written informed consent and received financial compensation for participation.

Measures

Emotional Stroop Task

The preconscious attentional processing of happy and angry faces was assessed using a masked pictorial emotional Stroop task. Facial stimuli of 10 different individuals (five males, five females) were taken from Ekman and Friesen's Pictures of Facial Affect (Ekman & Friesen, 1976), each displaying a neutral, a happy and an angry expression. The facial stimuli were presented for 14 ms. Immediately after the stimulus presentation the pictures were replaced by a masking stimulus. This procedure was extensively piloted in the laboratory of Van Honk and colleagues (Van Honk et al., 1998, 2000), who established an objective threshold for the recognition of emotional expressions for the displays. These pilots indicated that a 30 ms masking interval effectively

precluded recognition of the emotional valence of targets in every subject (Van Honk et al., 1998, 2000; Putman et al., 2004; Hermans et al., 2006; Roelofs et al., 2007). The masking stimuli consisted of randomly cut, reassembled and rephotographed pictures of faces. At each trial, the stimulus and mask were presented in the same color (red, green or blue), and participants were instructed to vocalize this color. Upon vocal response initiation, the presentation of the masking stimulus was terminated. After a random inter-trial interval (2-4 s) new trials started with a 750 ms lasting fixation point.

Table 2.1 Patients' and controls' demographic characteristics, DSM-IV axis I comorbid psychopathology and rates of reported interpersonal traumas and seizure characteristics.

Variable	Patients (N = 19)	Controls (N = 20)	Statistics
Age (SD) in years	27.6 (7.3)	22.1 (4.2)	$t(28.51)=2.85$, $p<.01$
Number of women	15	18	$X^2(1) = 0.91$, $p=.34$
using contraceptives[1]	6	10	$X^2(1) = 0.51$, $p=.48$
luteal menstruation cycle[2]	7	8	$X^2 (1) = 0.14$, $p=.71$
Educational level			$X^2(1) = 2.51$, $p=.11$
primary and secondary	15	11	
higher	4	9	
Comorbid psychopathology			
none	4	20	
mood disorder	4		
anxiety disorders			
panic disorder	2		
agoraphobia	4		
social phobia	3		
generalized anxiety disorder	4		
obsessive compulsive disorder	1		
post traumatic stress disorder	1		
somatoform disorders			
pain disorder	4		
somatization disorder	1		
Subjects reporting psychotrauma			
any interpersonal trauma	17	2	$X^2(1)=24.63$, $p<.001$
sexual	14	1	$X^2(1)=19.42$, $p<.001$
emotional	14	2	$X^2(1)=16.33$, $p<.001$
physical	12	1	$X^2(1)=14.83$, $p<.001$
Seizure characteristics	21.1 (7.9)		
age (SD) at onset in years	6.5 (7.4)		
disease duration (SD) in years	27.8		
frequency per 4 weeks (SD)	(30.2)		

[1]use of contraceptive was unknown in one patient; [2]menstruation cycle was indeterminable in two patients and one control.

A total of 30 happy, 30 angry and 30 neutral faces were presented in a random order with the restriction that the same color was never repeated more than twice consecutively (Van Honk et al., 1998; 2000; Putman et al., 2004; Hermans et al., 2006; Roelofs et al., 2007). The main outcome variable in the emotional Stroop task is the attentional bias score for emotional facial expressions, which is based on correct responses only and calculated on basis of interference scores, by subtracting the mean individual color-naming latencies of neutral faces from the individual mean color-naming latencies of emotional faces. A positive attentional bias score, indicating slower color-naming to emotional faces as compared to neutral faces, is interpreted as a vigilant response, whereas a negative attentional bias score, indicating faster color-naming to emotional faces as compared to neutral faces, is interpreted as an avoidant response (e.g. Mathews & MacLeod, 1994; Van Honk et al., 1998, 2000; Putman et al., 2004). In addition, error rates were registered for each group, condition and facial expression separately.

To maximize the quality of the voice key registration, the subjects were instructed to speak loudly and clearly, to keep their mouths open during the task, to avoid smacking their lips or coughing before responding and to avoid correcting their answers in case they had already started vocalizing an erroneous response. All instructions were rehearsed in a practice phase of nine stimulus presentations in which only masks were used (i.e. without facial stimuli).

Awareness check. To ascertain that subjects remained unaware of the facial expressions in the Stroop task, the efficacy of the masking procedure was checked by means of a separate awareness check administered at the end of the experiment. During this three-alternative, forced choice, happy- angry-neutral recognition procedure, a random set of 30 masked faces was shown to the subjects. In advance of the test the subjects were told explicitly that the set contained 10 happy, 10 neutral and 10 angry faces. Participants were instructed to indicate (or guess), whether the presented picture contained a neutral, happy or angry expression by pushing the corresponding button (see also Van Honk et al., 1998; 2000; Putman et al., 2004; Hermans et al., 2006; Roelofs et al., 2007).

Stroop Color-Word Task

Attentional processing of neutral stimuli was assessed using a computerized Stroop-color-word task (Stroop, 1935). Our version consisted of two series. In the first 'congruent' series four bars in the colors green, blue, red and yellow were each presented six times in random order and subjects were instructed to name the color of the bar as quickly as possible. The second 'incongruent' series of stimuli consisted of a total of 48 color words presented in a color different from the meaning of the word (e.g. the word red presented in green print). Participants were instructed to name the color of the print as quickly as possible. Each trial was presented centrally and presentation of the stimuli was terminated upon vocal response initiation. After a random inter-trial interval (2-4 s), new trials started with a 750 ms lasting fixation point. All instructions were practiced in a practice phase and, preceding the first series, each of the four colored bars was presented once. In order to give participants a chance to adjust to the instructions of the second 'incongruent' series, 12 practice trials preceded these series.

Naming the color of the print when the meaning of the word is an incongruent color, results in prolonged color-naming latencies compared to the color-naming latencies of the colored bars. This effect, known as Stroop interference, is calculated by subtracting the color-naming latencies of the first series from those of the second 'incongruent' series. This classic Stroop interference is consistently found and is explained by the costs for subjects to suppress a concurrent (automatic) competing response (for a comprehensive review see MacLeod, 1991). Details concerning validity and reliability have been described elsewhere (e.g. Strauss et al, 2005a; Alvarez & Emory, 2006).

Emotional, physical and sexual trauma

Emotional, physical and sexual traumas were measured by means of the Traumatic Experiences Checklist (TEC), a 26-item self-reported questionnaire with good reliability and validity (Nijenhuis et al., 2002). The scores for the presence of both emotional trauma (emotional neglect and emotional abuse in various settings) and sexual trauma (sexual harassment and sexual abuse in various settings) are based on six items. The scores for the presence of physical abuse in various settings are based on three items. All items are preceded by the phrase: *"Did this happen to you?".* An example of a sexual abuse item is:

"Sexual abuse (unwanted sexual acts involving physical contact) by your parents, brothers, or sisters". For all three types of interpersonal trauma a dichotomous score (*yes/no)* was calculated.

Trier Social Stress Test

This psychological challenge test consists of an anticipation period, a video- and audio-taped job application speech and a mental arithmetic task in front of a two-individual audience. The Trier Social Stress Task (TSST) takes 15 minutes, and has been found repeatedly to induce significant endocrine and cardiovascular responses in 70 – 80% of the participants (for a detailed description see Kirschbaum et al., 1993). In a review paper on acute laboratory stressors, the TSST was found to be the strongest elicitor of cortisol elevations (Dickerson and Kemeny, 2004). To ensure that stress levels remained high during the second administration of both Stroop tasks, the audience remained in the room after the TSST. After this, the audience left the room and subsequently returned for a short debriefing.

Physiological and subjective measures

To test the effectiveness of the stress-induction, several physiological and subjective stress measures were conducted as a manipulation check. With the exception of heart rate, all physiological and subjective stress-measures were obtained at 11 assessment points over a 200-minute period, at respectively -60, -40, -20, 0, (rest) +20, +40, (stress) +60, +80, + 100, +120 and +140 (recovery) minutes with reference to the start of the stressor. All assessments were performed between 1.15 and 5 pm. See also Figure 2.1.

Cortisol. Salivary (free) cortisol is a good indicator of glucocorticoid activity with the advantage (over blood cortisol samples) of stress-free (noninvasive) sampling. This method is therefore recommended in stress research where reliable 'baseline-to-stress' comparisons are essential (Kirschbaum et al., 1993; Kirschbaum & Hellhammer, 1994). Saliva samples were obtained using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Saliva 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, Switzerland), as described elsewhere (Van Aken et al., 2003).

Systolic (SBP) and diastolic blood pressure (DBP). SBP and DBP were measured from the nondominant arm using an automatic electronic digital blood pressure monitor, the Omron R5-I (Omron, Hoofddorp, The Netherlands) which could be initiated manually. This device fulfilled the validation criteria of international guidelines for both systolic and diastolic blood pressure (for more information see Omboni et al., 2007). Because of technical problems, both SBP and DBP data are missing for one patient and one control subject.

Heart rate (HR) and Heart rate variability (HRV). After the first sequence of physiological and subjective assessments, HR was continuously measured by the Ambulatory Monitoring System (AMS; version 4.6. TD-FPP, Vrije Universiteit, Amsterdam, The Netherlands). This device has been used extensively and details of its reliability, validity aspects and recording methodology have been published previously (De Geus et al., 1995; Willemsen et al., 1996). In the present study the electrocardiogram signal was recorded using disposable pre-gelled Ag–AgCl electrodes (ConMed, New York, USA) that were placed at the jugular notch of the sternum, 4 cm under the left nipple and at the lateral right side. Using this three-electrode configuration only the inter-beat interval time series were available for analysis. The device detects the R-wave of the electrocardiogram and records the time in ms (with 1 ms resolution). From the raw interbeat intervals the device derives and stores 30-s averages of HR (in beats per minute) and root mean-square of successive differences of interbeat intervals (in milliseconds: RMSSD), which was used as an index of HRV. The RMSSD has been shown to be a reliable index of cardiac parasympathetic influence and is recommended as a measure of vagally-mediated HRV for its simplicity (Task Force Guidelines, 1996; Thayer & Brosschot, 2005). Both HR and HRV were averaged per phase separately resulting in an average for the baseline period, for the stress condition and for recovery. Due to technical problems, both HR and HRV data are missing for one patient and two control subjects.

Subjective anxiety. Participants rated their subjective anxiety on a visual analogue scale, ranging from 0 (not anxious) to 10 (extremely anxious), at each assessment point.

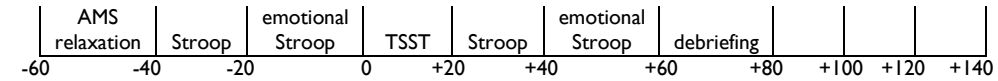

Figure 2.1. Outline of the experiment. Assessment points (in minutes with reference to the onset of the Trier Social Stress test: TSST) of the physiological and subjective stress parameters. AMS, Ambulatory Monitoring System.

Procedure

On the test day, participants arrived about 2 h before the first physiological assessments took place and more than 2 h before the cognitive tasks were administered. Participants were submitted to a standard protocol to control for factors that may influence HPA-axis activity and hence cortisol activity (e.g. exercise, lunch). Participants were first screened for DSM-IV axis-I psychopathology (American Psychiatric Association, 1994) using the MINI (Sheenan et al., 1998). No later than 30 minutes after arrival, subjects had a light lunch (sandwiches and soft drinks). Half an hour later the DSM-IV screening was continued (if necessary), the TEC questionnaire was completed and subjects were interviewed briefly about their professional ambitions in preparation for the public speaking part of the TSST (although participants were unaware of the purpose of this interview). The participants were taken to the experimental room after a further 45 minutes. The outline of the experiment is presented in Figure 2.1.

Statistical analyses

For the emotional Stroop task, color-naming latencies outliers were filtered using a <150 and >1500 ms cut-off. For the correct responses, all color-naming latencies exceeding 2.5 SD from their cell mean were subsequently removed (cell defined by Condition, Group and Emotional expression of the faces). The remaining latencies were averaged for each individual over Condition and Emotional expression and attentional bias scores were calculated subsequently. For the color-word Stroop task the same procedure was followed, except that cells were defined by Condition, Group and Series (congruent/incongruent).

For both the emotional and color-word Stroop tasks, percentages incorrect responses were calculated per cell. For the awareness-check, percentages of correct responses were calculated and a nonparametric test was applied to determine whether the patients' and controls' percentage correct responses did not exceed chance level.

Physiological and subjective stress measures were post hoc averaged per experimental phase (baseline: -20 to 0 minutes), stress (20 to 40 minutes) and recovery (60 to 140 minutes).

Performance on the emotional Stroop and the color-word Stroop, as well as the effects of stress-induction on physiological and subjective stress measures, was tested using repeated measures Analyses of Variance (ANOVA rm). The relationship between attentional bias scores and trauma ratings was calculated using Pearson correlations. All statistical analyses described employed a two-tailed alpha of 0.05.

Results

Manipulation checks

Stress-induction. To check whether the stress-induction was successful, separate two-way ANOVAs rm for the physiological and subjective stress measures were conducted with Group (Patients, Controls) as between-subject factor and Condition (baseline, stress, recovery) as within-subject factor. The results showed a significant main effect of Condition for cortisol ($F(2,36)=19.01$, $p<.001$), SBP ($F(2,34)=40.24$, $p<.001$); DBP ($F(2,34)=24.31$, $p<.001$); HR ($F(2,33)=35.44$, $p<.001$); HRV ($F(2,33)=6.07$, $p<.01$); and self-reported anxiety ($F(2,36)=34.61$, $p<.001$). With the exception of HRV, post hoc *F* tests for these measures demonstrated a relative increase during stress followed by a decrease during the recovery phase for all parameters (all *p*-values <.01), indicating that stress-induction was indeed successful. Group effects were present for only HRV (main effect of Group: $F(1,34)=5.30$, $p<.05$) and not for other subjective or physiological measures (all *p* values >.10). This finding indicated that patients had lower HRV than controls throughout the experiment. Post hoc testing demonstrated that this effect was particularly significant at baseline ($F(1,34)= 5.64$, $p<.05$) and during recovery ($F(1,34)= 4.93$, $p<.05$) but not during stress ($F(1,34)= 2.54$, $p=.12$), see Figure 2.2.

*Emotional Stroop Masking procedure***.** Chance performance in a three-alternative forced choice recognition check using 30 stimuli is 10 (33.3%) correct identifications per subject. Because of technical problems, the data of one of the 19 patients were not available. Of the total numbers of 540 trials, 178 (33.3%) were correctly recognized by

patients. All 20 controls completed the check (600 trials), of which 199 (33.2%) were correctly recognized. Nonparametric tests showed that there was no significant deviation from chance detection for the patients (p=.51) or the control group (p=.48). It can be concluded that masking was successful.

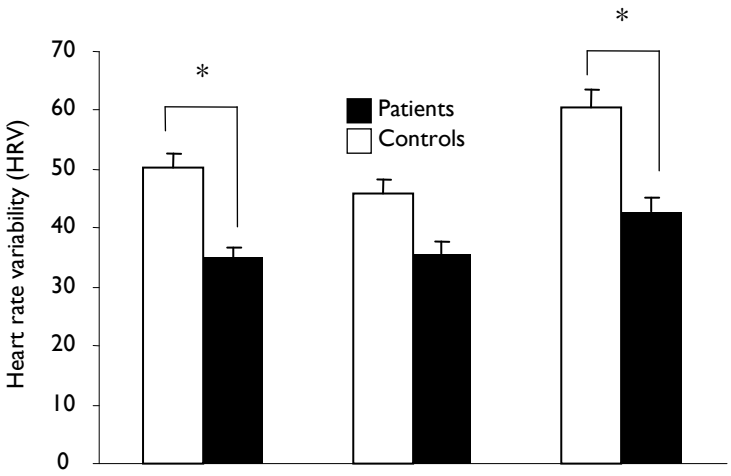

Figure 2.2. Patients' and controls' mean HRV rates (± SEM) during baseline, stress and recovery; * p < .05.

Attentional bias (AB) scores

Emotional Stroop

To investigate the AB scores for angry and happy faces at baseline and in the social stress condition, we conducted a three-way ANOVA rm for the AB scores, with Facial Expression (FE: happy, angry) and Condition (baseline, stress) as within-subject factors and Group (patients, controls) as between-subject factors. There were no main-effects for FE (F (1,37)=.96, p=.33), Condition (F (1,37)=.85, p=.36) or Group (F(1,37)=.07, p=.79), but there was a significant FE X Condition X Group interaction (F(1,37)=5.91, p < .05). Post hoc F tests to investigate this three-way interaction indicated that the FE X Group interaction was significant at baseline (F(1,37)=9.18, p<.005), but not during stress (F(1,37)=.02, p=.88). Further investigation of the results at baseline showed that PNES patients differed significantly from the controls in their response to angry faces (F(1,37)=4.18, p<.05) but not to happy faces (F(1,37)=1.07, p=.31). As illustrated in Figure 2.3, these results indicate that whereas patients showed a positive AB to angry

faces, controls showed a negative AB for these stimuli at baseline. These group differences disappeared in the social stress condition.

Finally, we checked whether the FE X Group interaction at baseline remained significant after controlling for age by entering Age as a covariate in the analysis. We found that this effect remained significant ($F(1,36)=5.12$, $p<.05$).

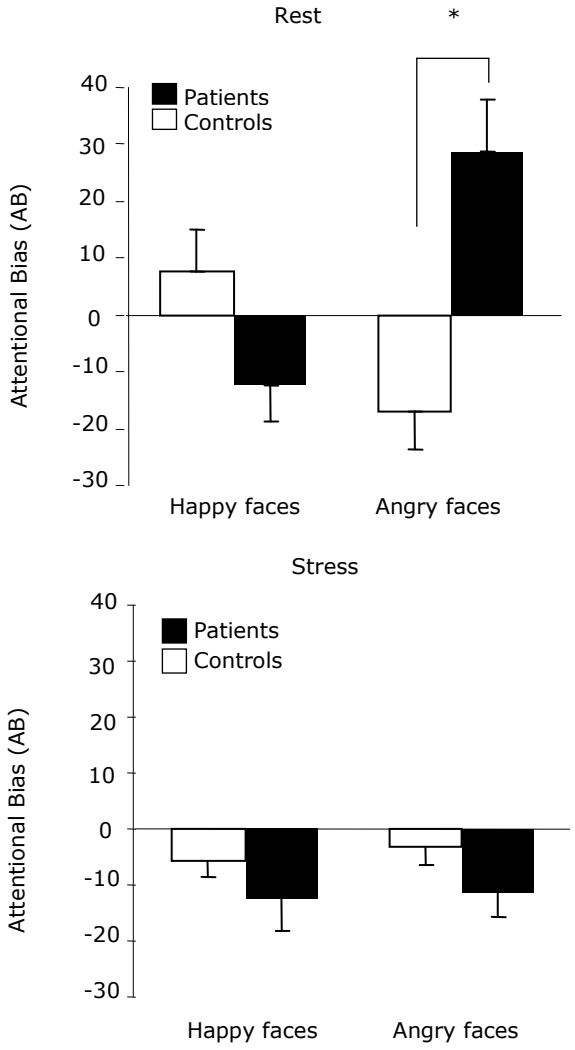

Figure 2.3. Mean attentional bias (AB) scores (color-naming latencies of emotional faces minus color-naming latencies for neutral faces) in ms (± SEM) for happy and angry faces in baseline and a social stress condition. A positive AB indicates vigilance; negative AB reflects avoidance; * $p < .05$.

Error rates. The FE x Condition x Group ANOVA rm for the error rates resulted in a main effect for Condition [(F(1,37)=15.62, p<.001): 2.7% (baseline) versus 1.6% (stress)] and FE [(F(2,36)=6.24, p<.01): 2.9% (angry); 2.1% (neutral); 1.4% (happy)]. Moreover, there was a significant interaction effect for Condition X Group (F(1,37)=12.78, p<.01), indicating that whereas patients performed less accurately at baseline (3.6%) as compared to stress [(1.4%); (F(1,18)=22.07, p<.001)], controls showed no such condition effect [(baseline = 1.8%; stress = 1.7%; (F(1,19)=.10, p=.76)].

Stroop Color-Word

To investigate the selective attention for neutral stimuli at baseline and during stress we conducted a two-way ANOVA rm for the Stroop interference scores, with Condition (baseline, stress) as within-subject factor and Group (patients, controls) as between-subject factors. There were no main effects for Condition (F (1,37)=1.04, p=.31) and Group (F (1,37)=.07, p=.80), and no interaction effects for Condition X Group (F (1,37)=.00, p=.98), indicating that selective attention for neutral stimuli was unaffected in patients with PNES.

Error rates. The Condition x Group ANOVA rm for the error rates revealed no significant main effects, but there was a significant Condition X Group interaction (F(1,37)=6.19, p<.05), indicating that whereas patients were less accurate at baseline (7.8%) compared to stress [(4.3%); (F(1,18)=9.56, p<.01)], controls showed no such condition effect [(F(1,19)=1.27, p=.27; baseline=3.7%; stress=5.9%)].

AB and trauma reports

Because there were only effects for the emotional and not for the neutral Stroop task, correlations with trauma reports were only calculated with respect to the emotional Stroop task. The patients' positive AB for angry faces at baseline was positively correlated to the presence of sexual trauma reports (Pearson's point correlation: r=.46, p<.05), indicating that patients' increased sexual trauma reports were associated with a positive AB for angry face stimuli on the masked emotional Stroop task. The correlation between the patients' positive AB angry faces at baseline and physical abuse was in the same direction but did not reach significance (r=.39, p=.10). There were no such effects for emotional trauma (r=.18, p=.45) and no such effects for the control subjects (all p values >.30).

Discussion

In this study, PNES patients and matched controls did not differ in their performance on a *neutral* (and unmasked) Stroop task, but they showed significant differences in the processing of *emotional* stimuli on a masked pictorial Stroop task. Whereas the healthy controls displayed a negative attentional bias (AB) for angry faces, patients showed a positive AB for these social threatening stimuli, indicating that on a preconscious level of processing, patients were vigilant for social threat stimuli. In addition, this increased threat vigilance was related to self-reported trauma in patients with PNES. Below we will describe these results in detail and discuss their implications.

The finding that patients with PNES reported more traumatic events than controls fits with the generally found high trauma rates in patients with PNES (e.g. Betts & Boden, 1992; Bowman, 1993; Moore & Baker, 1997; Kuyk, et al., 1999; Fiszman et al., 2004; Sharpe & Faye, 2006) and conversion disorder in general (Roelofs et al., 2002). Most importantly, self-reported sexual trauma was related to the positive AB for angry faces in the patient group but not in controls. This relationship between threat vigilance and trauma reports shows an interesting parallel with findings in patients with PTSD to trauma-specific threat stimuli (for a review see McNally, 1998; Buckley et al., 2000). In PTSD patients, such vigilance for trauma-related stimuli is considered as a tendency to constantly scan the environment for any signs of potential threat (Buckley et al., 2000) or it could reflect an impaired suppression of trauma information once it is activated (McNally, 1998). A similar positive AB for preconsciously presented angry faces, using the same masked pictorial Stroop task, was found in traumatized subjects with Dissociative Identity Disorder (Hermans, et al., 2006), which was interpreted as indicating a state of hypervigilance. The finding of increased allocation of attentional resources to social threat in the current study may similarly reflect a state of hypervigilance, an interpretation that is supported by the finding that patients with PNES showed decreased heart rate variability (HRV) throughout the experiment. Decreased HRV is associated with increased arousal and anxiety and was previously found in patients with anxiety disorders, such as panic disorder (Friedman & Thayer, 1998), generalized anxiety disorder (Thayer et al., 1996) and PTSD (Cohen, et al. , 1999) and has

been suggested as being associated with poor emotion regulation (Ruiz-Padial et al. 2003) and a negativity bias (Thayer & Brosschot, 2005). It is interesting to relate these findings to previous findings of repressive coping styles in PNES (e.g. Frances et al., 1999; Goldstein et al. 2000). Cognitive vigilance and avoidance are considered as ways of coping in the face of threat (e.g. Calvo & Eysenck, 2000; Hock & Krohne, 2004) and so-called repressors are characterized by an initial disproportionate engaging in threat processing, followed by an avoidance of threat processing and high physiological arousal (Calvo & Eysenck, 2000). Future studies should investigate whether the threat-vigilance identified in the present study may be associated by subsequent avoidance, for example by using a modified dot-probe paradigm (see Mogg et al. 1997; Bögels & Mansell, 2004). Such investigation is particularly relevant because seizure reduction or cessation is generally associated with more active coping strategies in patient with PNES (Bodde et al.; 2007, Kuyk et al., 2008) and it may contribute to fine tune psychological treatment of PNES. In contrast, the (early) avoidant coping style exhibited by our healthy controls in the face of threat is considered as an adequate manner to avoid injury and unnecessary energy loss (Sapolsky, 1990; Van Honk et al., 2000).

In the present study, an increase of subjective and physiological stress parameters during stress in both patients and controls suggested that the stress-induction by means of the Trier Social Stress Test was successful. The group difference in attentional processing of social threat stimuli reported for the baseline condition was no longer present when subjects were tested in the context of social stress. Although this finding was in contrast to our predictions derived from Bendefeldt et al. (1976), this result is in agreement with earlier studies in patients with PTSD (Constans et al, 2004) and social phobia (Amir et al., 1996) in which patients exhibited a positive AB for threat words in a emotional Stroop task at baseline, which was suppressed in anticipation of a stressor. Because a positive AB for angry faces is often taken as indicating hypervigilance for signs of social threat, the fact that this effect disappeared during stress may be related to the unambiguousness of the social stress context, which makes an AB towards social threat stimuli in the emotional Stroop task simply redundant. Such interpretation is supported by Pessoa et al. (2002) who found that processing of emotional stimuli in a highly demanding environment did not lead to an activation of the amygdala. It was previously argued that

in a highly demanding environment all available attentional resources are focused on the environment, not on the cognitive task, resulting in reaction patterns that are independent of the emotional valence of the emotional stimuli (Lavie, 1995). In our study the disappearance of the patients' positive AB to angry faces in the social stress condition may reflect an allocation of all attentional resources towards the socio-evaluative threat of the audience in this condition. Alternatively, it is possible that patients put more effort into complying with the task demand, in the context of social stress, resulting in a suppression of the AB for angry faces. The fact that patients made fewer errors in both Stroop tasks during stress, as compared to baseline, supports this notion, although this latter finding could also reflect a possible learning effect.

Patients and controls did not differ with respect to their basal and stress-induced cortisol levels. Although these findings are suggestive of a normal stress-reactivity of the HPA-axis in PNES, it should be noted that the currently used stressor was not specific for this disorder. In the context of trauma-related disorders the use of personalized trauma scripts may constitute a more relevant or specific stressor, yielding different results (e.g. Elzinga et al., 2003).

When evaluating these results some strengths and limitations of the present study should be considered. A strong point is that all participating patients were diagnosed using the golden standard: an ictal video-EEG registration of a typical seizure in order to confirm the absence of epileptiform activity during a seizure (Reuber & Elger, 2003), making the diagnosis of PNES maximally reliable. Secondly, the fact that all participating patients were unmedicated rules out the possibility that the altered cognitive processing in our patients was the result of medication effects. As a consequence however, we cannot automatically generalize these results to PNES patient who are on medication. Thirdly, previous studies on neuropsychological functioning in patients with PNES were solely focused on the cognitive processing of nonemotional information (see Cragar et al., 2002 for a review). This is the first study investigating the cognitive processing of emotional stimuli in PNES. Facial expressions constitute important signals of threat or appeasement in the social environment (Öhman, 1986). Several neuroimaging studies have shown that viewing angry faces activates limbic structures, the amygdala in particular (for an overview see Adolphs et al., 2002; McClure et al., 2004; Strauss et al., 2005b), supporting the relevance of

these stimuli in the study of stress-related disorders and the role of interpersonal trauma, in particular. Finally, the use of a masked Stroop task has the advantage that the subjects do not consciously perceive the stimuli, which was confirmed by the results from our awareness-check. This makes it unlikely that subjects exerted strategic effort to control AB effects (e.g. MacLeod & Hagan, 1992; Van den Hout et al. 1995; Williams et al., 1996; Putman et al., 2004) and makes the findings less vulnerable to uncontrollable subject factors.

A limitation of the present study is the lack of a clinical control group, making it difficult to state the specificity of the effects for the group with PNES and to exclude the possibility that the altered AB was mediated by comorbid psychopathology. However, in this respect it is relevant to mention that application of exactly the same masked emotional Stroop Task in patients with social phobia resulted in opposite results; these patients allocated their attention away from the social threatening stimuli (E. Hermans, unpublished data). Despite this limitation, our data provide the first evidence linking interpersonal trauma with altered emotional processing in patients with PNES and give rise to several interesting questions for future research exploring the possible psychiatric mechanisms associated with PNES. For example, although we found clear results on the processing of masked emotional Stroop stimuli in our patient group, it remains to be tested whether the same findings hold for unmasked threat stimuli. Secondly, as stated earlier, it would be very interesting to replicate the present study by inducing stress using a more relevant/specific stressor, namely personalized trauma scripts or a physiological stressor. Thirdly, considering that PNES is a rather heterogeneous group with respect to PNES characteristics, it would be interesting to investigate the effects of, besides psychotrauma, different PNES presentations (see e.g. Selwa et al., 2000) and comorbid psychopathology on the attentional processing of threatening stimuli. This is particularly relevant to gain insight into the possible different underlying mechanisms in the diverse semiology of PNES. Lastly, neuroimaging studies in PNES are needed to investigate which brain structures are involved in the processing of altered emotional information in neutral and stress conditions.

In conclusion, the present study showed impaired emotional information processing in patients with PNES. Compared to healthy controls, patients showed increased vigilance for masked angry faces. This preconscious AB for angry faces was significantly correlated to self -

reported sexual trauma rates and probably reflects a state of hypervigilance. This interpretation is further supported by the finding of decreased HRV in patients with PNES, which was previously related to increased arousal/anxiety and poor emotion regulation. Given these results, further experimental research, investigating the relationship between attention, trauma, stress and coping in patients with PNES seems promising to gain additional insight in possible neuropsychiatric mechanisms underlying this disorder with the ultimate purpose of improving (psychological) care for and treatment of this invalidating disorder.

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CHAPTER 3

Basal hypercortisolism and trauma in patients with Psychogenic Non Epileptic Seizures

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Abstract

Purpose. Several studies have indicated that Psychogenic Non Epileptic Seizures (PNES) are associated with psychological trauma, but only a few studies have examined the associations with neurobiological stress systems, such as the Hypothalamus-Pituitary-Adrenal (HPA)-axis and its end-product cortisol. We tested several relevant HPA-axis functions in PNES patients and related them to trauma history.

Methods. Cortisol awakening curve, basal diurnal cortisol and negative cortisol feedback (using a 1 mg Dexamethasone-Suppression-Test) were examined in 18 PNES patients and 19 matched healthy controls (HCs) using saliva cortisol sampling on two consecutive days at 19 time-points. Concomitant sympathetic nervous system (SNS) activity was assessed by analyzing saliva alpha-amylase (sAA).

Results. Patients with PNES showed significantly increased basal diurnal cortisol levels compared to HCs. This effect was driven mainly by patients reporting sexual trauma who showed a trend towards higher cortisol levels as compared to patients without a sexual trauma report. Importantly, the increased basal diurnal cortisol levels in patients were not explained by depression, medication, smoking, or by current seizures or group differences in SNS activity.

Discussion. This is the first study showing that basal hypercortisolism in patients with PNES is independent from the acute occurrence of seizures. In addition, basal hypercortisolism was more pronounced in traumatized patients with PNES as compared to nontraumatized patients with PNES. These findings suggest that HPA-axis activity provides a significant neurobiological marker for PNES.

Introduction

Several studies have indicated that Psychogenic Non Epileptic Seizures (PNES) are associated with a history of psychological trauma, such as sexual and physical abuse (for reviews see e.g. Fiszman et al., 2004; Sharpe & Faye, 2006, Roelofs & Spinhoven, 2007). Only few studies have investigated the association of PNES with neurobiological stress systems, such as the Hypothalamus-Pituitary-Adrenal (HPA)-axis with cortisol as its end-product. The majority of these studies focused on the effects of seizure-like activity on cortisol levels and found mostly increased cortisol levels in PNES patients (as well as in confirmed epilepsy patients) related to seizures (e.g. Mehta et al., 1994; Tunca et al., 2000). So far, only two studies have investigated *basal* activity of the HPA-axis in PNES and the results are conflicting. Tunca et al. (1996) did not find increased basal cortisol levels in a sample of 25 patients with conversion disorder (including 20 patients with PNES) compared to healthy controls (HCs) but did find decreased cortisol suppression after dexamethasone administration. In contrast, in a sample of eight patients with PNES, Tunca et al. (2000) later observed increased morning serum cortisol levels at baseline (an average time interval of 18 h had elapsed since the last seizure). In addition, we found no indications for increased *stress-induced* cortisol levels in patients with PNES but, in line with previous notions of increased *basal* activity of physiological stress-related systems in patients with PNES, we found decreased levels of basal heart rate variability (Bakvis et al., 2009a), often taken as an indication of hyperarousal (see e.g. Thayer & Brosschot, 2005, for a review). In summary, previous accounts on HPA-axis activity in patients with PNES have shown mixed results and none of the previous studies has investigated the relationship between interpersonal trauma and HPA-axis activity in patients with PNES.

In an attempt to establish a neurobiological marker associated with PNES, the present study was designed to test several relevant HPA-axis functions, including Cortisol Awakening Response (CAR), basal diurnal cortisol and negative cortisol feedback (using a Dexamethasone-Suppression-Test, DST) in patients with PNES and to relate eventual findings to the occurrence of seizures and trauma history. In contrast to previous studies on basal HPA-axis activity in patients with PNES, we used a stress-free noninvasive method for measuring cortisol (saliva

instead of blood: Kirschbaum et al., 1993; Kirschbaum & Hellhammer, 1994), we tested only those patients whose diagnosis was based on an ictal electroencephalography (EEG)-video registration of a typical seizure and we controlled for current depression, use of psychotropic medication, smoking and menstrual cycle. In addition, we checked whether eventual alterations in HPA-axis activity were accompanied by concomitant group differences in activation of the sympathetic nervous system (SNS) through salivary alpha-amylase (sAA), which is indicative of acute stress (for reviews see Granger et al., 2007; Rohleder & Nater, 2009).

Based on Tunca et al. (1996, 2000), we predicted that we would find increased HPA-axis activity (as evidenced by increased basal diurnal cortisol levels, increased CAR and increased post-DST cortisol) in patients with PNES compared to HCs. Second, in accordance with an extensive body of literature linking negative life experiences to long lasting increases in HPA-axis activity in both animals (e.g. Sapolsky, 1997; Anisman et al., 1998) and humans (for reviews see e.g. Yehuda et al., 2006; Gunnar & Quevedo, 2008), and, in particular, in accordance with previous findings suggesting that sexual trauma was related to increased threat vigilance in patients with PNES (Bakvis et al., 2009a), we hypothesized that the expected increased HPA-axis activity in patients with PNES would be related to sexual trauma.

Methods

Participants

From March 2005 until April 2007, 20 patients with PNES, who had been admitted to a tertiary epilepsy centre, were recruited by the attending neurologists. Inclusion criteria were: (1) diagnosis of PNES based on an ictal video-EEG recording of a typical seizure, (2) PNES characterized by complete or partial loss of consciousness (specified as an ictal diminished or loss of adequate responsiveness or post-ictal memory impairments of the ictal event), (3) the occurrence of at least two seizures in the year prior to the experiment, (4) no history of concomitant epileptic seizures, (5) no comorbid neurological disease diagnosis, and (6) no diagnosis of endocrine disorder(s). Two of the 20 patients had to be excluded because of the occurrence of several seizures during the test days and consequently missing values. The

remaining 18 patients (7 males, 11 females) had a mean age of 31.6 (*SD*=10.8) years. Demographic data, use of contraceptives, use of psychotropic medication, smoking status, current comorbid *DSM-IV* axis I diagnoses (assessed using the MINI: Mini-International Neuro-psychiatric Interview, Sheenan et al 1998; Van Vliet & De Beurs, 2007), self-reported interpersonal traumatic experiences, seizure characteristics and the occurrence of seizures an hour preceding sampling on test days are provided in Table 3.1. The healthy control (HC) group was recruited through advertisements in local newspapers. Inclusion criteria were: (1) no psychiatric diagnosis, (2) no medical disease diagnosis, (3) no neurological disease diagnosis, and (4) no use of medication.

One of the 20 HCs who participated in this study was removed from analyses post hoc, due to extremely high cortisol levels indicative of endocrinopathy and was advised to contact her physician for assessment. The remaining 19 HCs (10 males, 9 females) had a mean age of 35.1 (*SD*=13.5) years. Patients and HCs did not differ with respect to age, gender and use of contraceptives (Table 3.1). Slightly more patients with PNES smoked and as expected, more patients with PNES used psychotropic medication. Furthermore, PNES patients reported higher rates of sexual trauma and overall interpersonal trauma compared to the control group (see Table 3.1 for statistics).

Measures

Cortisol and alpha-amylase

Saliva samples for cortisol assessments were obtained using Salivette collection devices with a cotton roll (Sarstedt, Rommelsdorf, Germany). In total 19 samples were taken over two consecutive days. The salivary samples on day 1 were taken at the time of awakening and 15, 30, 45 and 60 minutes afterwards (Cortisol Awakening Response: CAR) and at 10.00h, 12.00h, 14.00h 16.00h, 18.00h, 20.00h and 22.00h (basal diurnal cortisol). Participants were instructed to take a tablet of dexamethasone (1 mg) at 23.00 h on day 1. The following day participants collected salivary samples again at the time of awakening and 15, 30, 45 and 60 minutes afterwards and at 16.00 h and 22.00 h. (post-DST cortisol). Saliva 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, Switzerland), as described elsewhere (Van Aken et al., 2003).

Table 3.1. Demographic and clinical characteristics for 18 patients with PNES and 19 healthy controls.

Variable	Patients (N = 18)	Controls (N = 19)	Statistics
Mean age (SD) in years	31.6 (10.8)	35.1 (13.5)	$t(35)=.84$, $p=.400$
Number of women	11	9	$X^2(1) = .70$, $p=.402$
using contraceptives	6	8	$X^2(1) = .30$, $p=.582$
Smokers	6	1	$X^2(1) = 4.75$, $p=.029$
Taking psychotropic medication	9	0	$X^2(1) = 12.55$, $p<.001$
paroxetine	4		
risperdon	1		
fluoxetine	2		
oxazepam	1		
sertraline	1		
temazepam	1		
valproic acid	1		
flurazepam	1		
citalopram	1		
Participants reporting interpersonal trauma	11	5	$X^2(1)=4.56$, $p=.033$
sexual	7	2	$X^2(1)=4.04$, $p= .044$
emotional	8	4	$X^2(1)=2.31$, $p=.129$
physical	6	3	$X^2(1)=1.55$, $p=.214$
Current comorbid psychopathology	11	0	
none	7	19	
mood disorder	3		
anxiety disorders			
panic disorder	3		
agoraphobia	3		
social phobia	3		
generalized anxiety disorder	3		
obsessive compulsive disorder	1		
somatoform disorders			
pain disorder	1		
somatization disorder	1		
hypochondrias	1		
Mean age (SD) at onset seizures	26.6 (12.3)	-	
Disease duration in years (SD)	5.3 (5.5)	-	
Number of patients reporting seizures 1hr preceding sampling			
day 1			
cortisol awakening response	0	-	
basal diurnal cortisol	3	-	
day 2	1	-	

To check whether eventual alterations in HPA-axis activity were accompanied by concomitant group differences in SNS activation, alpha-amylase levels were also analysed from these saliva samples. Alpha-amylase levels have been shown to reflect SNS activity (for reviews see Granger et al., 2007; Rohleder & Nater, 2009.). Biochemical analysis of sAA was performed using a kinetic maltotrioside method (CNP-G3; DiaSys Diagnostic Systems, Holzheim, Germany) at 405 nm for serum on a Vitalab Selectra (Merck, Darmstadt, Germany) after dilution of saliva with saline (25 ul saliva plus 10 ml saline). The detection limit for the method in serum (or diluted saliva) was 2 U/L. The intra-assay variability coefficient was 1.6% at 411 U/L (N=10); the inter-assay variability coefficient was smaller than 2.8% in the range of 117-652 U/L (N=30).

Emotional, physical and sexual trauma

Emotional, physical and sexual traumas were measured by means of the Traumatic Experiences Checklist (TEC), a 26-item self-reported questionnaire with good reliability and validity (Nijenhuis et al., 2002). The scores for the presence of both emotional trauma (emotional neglect and emotional abuse in various settings) and sexual trauma (sexual harassment and sexual abuse in various settings) are based on six items. The scores for the presence of physical abuse in various settings are based on three items. For all three types of interpersonal trauma a dichotomous score (*yes/no)* was calculated.

Procedure

Candidate participants were invited for an initial informative session and subsequently to select dates appropriate for testing. Because of the influence of estradiol on the HPA-axis (Van Veen et al., 2008) women using oral contraceptives had to be tested in their gap-week. Women not on oral contraceptives had to be tested in the follicular phase of their menstrual cycle and were, therefore, instructed to record one menstrual cycle and to contact the researcher when the second menstruation had started to plan the definite dates for testing. After the test days were planned and participants had provided informed consent, all participants were administered a semi-structured diagnostic interview by author P.B. and two trained psychology master students, to screen for DSM-IV axis I disorders (American Psychiatric Association, 1994; assessed using the MINI: Mini-International Neuropsychiatric

Interview, Sheenan et al., 1998; Van Vliet & De Beurs, 2007). In addition, the trauma questionnaire was administered. Next, participants were informed about the necessity of strictly following the procedures and the time schedule for saliva sampling to obtain valuable data. They were instructed to contact the principal investigator to postpone the test in case of a febrile illness within 3 days before the test. In addition, they were asked not to perform strenuous physical exercise and to avoid stressful situations as much as possible on these 2 days. Finally they had to write down their activities and the occurrence of seizures during the hour before saliva sampling on the test days. For each saliva sample, participants were asked to place the cotton wad from a Salivette saliva collection tube in their mouth until the cotton roll was saturated, and to subsequently keep the tube containing the wad prelabeled with date and time in the refrigerator. For the awakening samples subjects were instructed to start saliva sampling immediately at awakening. Subjects were instructed to complete the early morning sampling before breakfast and possible medicine intake to avoid contamination of saliva with food or drinks. They were asked not to brush their teeth before completing the saliva sample 60 minutes after awakening. In addition, participants were instructed not to eat, drink or smoke 15 minutes before sampling. All instructions were given both verbally and in writing.

Participants received financial incentives for their participation in this study. The protocol was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC).

Statistical analyses

Outliers in cortisol were defined as values that deviated more than 2.58 standard deviations (*SD*s; i.e. the 99th percentile) from the group mean per assessment. For patients 0.9% and for HCs 1.1% of the total amount of cortisol samples were removed. Missing data including outliers (patients 1.2%; HCs 2.5%) were interpolated linearly by using the participant's preceding and following salivary cortisol values, and modeling the average curve from the participants' group over these values for that point in time. Subsequently, to normalize distributions, cortisol levels were subjected to natural log transformation before analyses. Separate repeated measures Analyses of Variance (ANOVA rm) were conducted for the CAR, for the basal diurnal cortisol and for

post-DST cortisol, each with Time (salivary time points) as within-subject factor and Group (patients, HCs) as between-subject factor. For the CAR and post-DST cortisol we controlled for the time of awakening (ToA) on day 1 and 2, respectively, by adding this variable to the analysis as a covariate. In the case of significant group effects, we controlled for depression, psychotropic medication and smoking by repeating the analysis for (1) a subgroup of patients without a current depression, (2) a subgroup of patients not on psychotropic medication, (3) nonsmoking participants, and (4) nonsmoking, nondepressed participants not on psychotropic medication. To test whether group differences in cortisol could be attributed to seizures in patients, analyses were repeated for those patients not reporting seizures one hour before saliva sampling. Finally, to investigate whether cortisol effects were particularly pronounced for those patients who reported sexual trauma, we conducted an additional three-group analysis with post hoc Least Significant Difference (LSD) analyses comparing PNES patients with and without sexual trauma reports and HCs without sexual trauma reports.

Alpha-amylase (AA) was investigated to test whether possible group differences in cortisol levels were accompanied by concomitant group differences in SNS activation. Outliers in basal diurnal alpha-amylase were defined as values that deviated more than 2.58 SD from the group mean per assessment. For patients 2.4% and for HCs 3.0% of the samples were removed. Missing data including outliers (patients 3.2%; HCs 3.0%) were interpolated linearly. To normalize distributions, AA levels were subjected to natural log transformation before analyses. An ANOVA rm was conducted for basal diurnal alpha-amylase with Time (salivary time points) as within-subject factor and Group (patients, HCs) as between-subject factor.

All statistical analyses described employed a two-tailed alpha of 0.05. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 16.0 for Windows.

Results

Cortisol Awakening Response (CAR)

To investigate possible group effects in the CAR, we conducted a two-way ANOVA rm for the morning cortisol levels with Time (five

assessment points from awakening until 60 minutes after awakening) as within-subject factor and Group (patients, HCs) as between-subject factor. Results showed a significant effect for Time ($F(4,32) = 6.13$, $p=.001$) but not for Group ($F(1,35)=1.21$, $p=.279$), or Time X Group ($F(4,32)=2.14$, $p=.099$). These effects for group did not alter when adding Time of Awakening on day 1 (ToA1) as a covariate to this analysis [Group ($F(1,33)=1.04$, $p=.315$); Time X Group ($F(4,30)=2.02$, $p=.116$); ToA1 ($F(1,33)=.15$, $p=.704$)]. Therefore groups did not differ significantly with respect to the CAR.

Post Dexamethasone-Suppression-Test (DST) cortisol

To investigate possible group effects in negative cortisol feedback by the DST, a two-way ANOVA rm was conducted for the post-DST cortisol levels with Time (seven assessment points from awakening until 60 minutes after awakening and at 16.00 h and 22.00 h) as within-subject factor and Group (patients, HCs) as between-subject factor was conducted. Results showed significant main effects for both Time ($F(6,30)=8.59$, $p<.001$) and Group ($F(1,35)=4.90$, $p=.033$, see Figure 3.1). The time X Group interaction was not significant ($F(6,30)=.16$, $p=.984$). These results for Group did not change when adding Time of Awakening on day 2 (ToA2) as a covariate to this analysis [Group ($F(1,33)=5.41$, $p=.026$); Time X Group ($F(6,28)=.15$, $p=.987$); ToA2 ($F(1,33)=.49$, $p=.488$)]. ToA2 was therefore excluded from the subsequent analyses. This main effect for Group remained significant when excluding patients with depression [(remaining N patients = 15; N controls = 19; Group ($F(1,35) = 4.90$, $p=.033$)]; remained a statistical trend when excluding patients taking psychotropic medication [(N patients = 9; N controls = 19; Group ($F(1,26)= 3.86$, $p =.060$)]; but did not remain significant when excluding smoking participants [(N patients = 12; N controls =18; Group ($F1,28)=1.01$, $p=.325$)]. Therefore we cannot conclude that post-DST cortisol levels were specifically affected for patients with PNES.

Basal Diurnal Cortisol

To investigate possible group effects in basal diurnal cortisol levels, we conducted a two-way ANOVA rm for the salivary basal diurnal cortisol with Time (seven assessment points from 10.00 h until 22.00 h) as within-subject factor and Group (patients, HCs) as between-subject factor. Results showed significant main effects for Time ($F(6,30)=22.60$,

p<.001) and Group[1] (F(1,35)=8.89, p=.005) and most importantly a significant Time X Group interaction (F(6,30)=3.07, p=.018, see Figure 3.1). The Time X Group effect for basal diurnal cortisol remained significant when repeating these analyses for (1) a subgroup of patients without a current depression [(N patients = 15; N controls = 19; Time X Group (F(6,27)=3.16, p=.018)]; (2) a subgroup of patients not taking psychotropic medication [(N patients = 9; N controls =19; Time X Group (F (6,21)=2.82, p=.036)]; (3) a subgroup without smoking participants [(N patients = 12; N controls =18; Time X Group (F(6,23)=3.24, p=.019)]. In addition, this effect even remained a statistical trend when repeating this analysis for the small subgroup of nondepressed, nonsmoking participants who were not using psychotropic medication [(N patients = 6; N controls =18; Time X Group (F(6,17)=2.34, p=.079)].

Post hoc F testing indicated, that PNES patients displayed higher basal cortisol at 12.00 h (F(1,35)=5.88, p=.021), 14.00 h (F(1,35)=6.21, p=.018), 16.00 h (F(1,35)=9.85, p=.003) , 18.00 h (F(1,35)=10.29, p=.003) and 20.00 h [(F(1,35)=11.63, p=.002); (other p>.106)].

To check whether these increased basal diurnal cortisol levels in patients with PNES were not related to current seizures, we repeated the latter tests for the 15 patients not reporting seizures one hour prior to sampling. Results showed that the group effects remained significant at all time points: 12.00 h (F(1,32)= 6.06, p=.019), 14.00 h (F(1,32)= 5.60, p=.024), 16.00 h (F(1,32)= 8.38, p=.007), 18.00 h (F(1,32)= 8.73, p=.006) and 20.00 h (F(1,32)=9.75, p=.004); (other p>.098)], indicating that the higher basal diurnal cortisol in patients with PNES were not attributable to current seizures.

[1] We found the same group effect when conducting an ANOVA with Area Under the Curve [AUC] with respect to ground [(AUCg); F(1,35)=10.37, p=.003)]; for more details see Pruessner et al., 2003, formula 2).

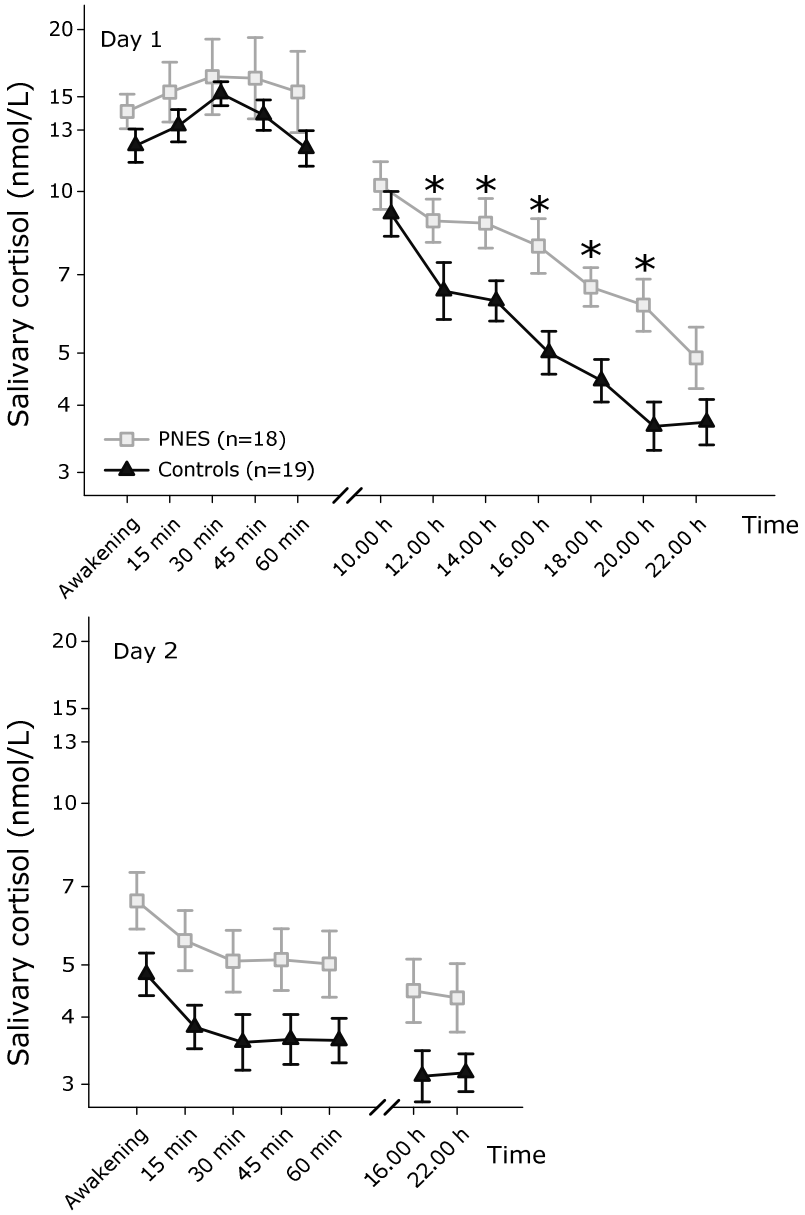

Figure 3.1. The upper panel shows the basal salivary cortisol concentrations of day 1 on a logarithmic scale. The lower panel shows the post-Dexamethasone-Suppression-Test (DST) salivary cortisol concentrations of day 2 on a logarithmic scale. Groups did not differ significantly on salivary Cortisol Awakening Response (CAR; at awakening, + 15 min., + 30 min., + 45 min., + 60 min.). Patients with PNES had higher basal diurnal cortisol levels (10.00 h, 12.00 h, 14.00 h, 16.00 h, 18.00 h, 20.00 h, and 22.00 h, see *). Post-DST cortisol levels (awakening, + 15 min., + 30 min., +45 min., + 60 min., 16.00 h, and 22.00 h) were increased in patients with PNES, but this group effect disappeared when controlling for smoking and psychotropic medication.

To test our second hypothesis that this effect would be particularly pronounced in PNES patients who experienced sexual trauma, an additional analysis for the five basal diurnal cortisol sample points on which groups differed (12.00 h, 14.00 h, 16.00 h, 18.00 h, and 20.00 h) was conducted. A two-way ANOVA rm with Time (five assessment points) as within-subject factor and Group (patients with sexual trauma (*N* =7), patients without sexual trauma (*N* =11) and HCs without sexual trauma (*N* =17, see Table 3.1) as between-subject factor showed a main effect for Group ($F(2,32)=9.11$, $p=.001$), and a statistical trend towards significance for Time X Group ($F(8,60)=1.90$, $p=.077$, see Figure 3.2). Post hoc LSD analyses indicated that the HC group had significantly lower basal diurnal cortisol rates as compared to patients with sexual trauma ($p<.001$); as well as patients without sexual trauma ($p=.021$). Furthermore, post hoc LSD analyses indicated a trend towards significance ($p=.067$) for the difference in basal diurnal cortisol between patients with and without a history of sexual trauma.

Thus, patients with PNES displayed heightened basal HPA-axis activity compared to HCs, and this effect was particularly pronounced in those patients reporting sexual trauma.

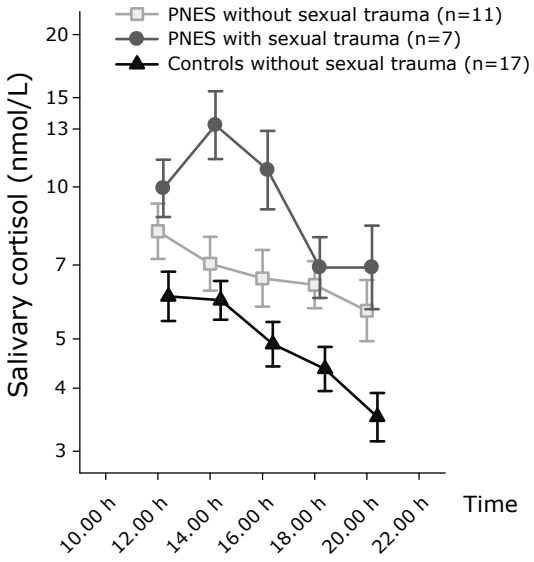

Figure 3.2. Basal diurnal salivary cortisol concentrations of day 1 on a logarithmic scale. Healthy controls (*N* =17*)* differed significantly from both patients with (*N* =7*)* and without sexual trauma (*N* =11*)* on basal diurnal cortisol levels. In addition, patients with sexual trauma showed increased cortisol levels as compared to patients who did not report sexual trauma.

Alpha-Amylase

In order to investigate whether the group effects in basal cortisol levels were associated with concomitant group effects in SNS activity, we conducted a two-way ANOVA rm for saliva basal diurnal alpha-amylase levels with Time (seven assessment points from 10.00 h until 22.00 h) as within-subject factor and Group (patients, HCs) as between-subject factors. Results showed that there was a significant main effect for Time ($F(6,30)=5.45$, $p=.001$), but that there were no significant effects for Group (all $p>.211$) indicating that patients' increased HPA-axis activity was not accompanied by concomitant group differences in SNS activation.

Discussion

The main purpose of the present study was to investigate HPA-axis activity in patients with PNES compared to age and gender matched healthy controls (HCs).

Patients' basal cortisol levels were augmented in the afternoon and evening. Previous reports of increased HPA-axis activity in patients with PNES also indicated higher levels of afternoon and evening cortisol (Tunca et al., 2000). In the latter study, however, the increased cortisol levels were related to seizures. Here, we show that the increased basal cortisol levels in patients with PNES occurred independent of the acute presence of seizures. In addition, we found that the increased basal cortisol levels could not be explained by factors such as increased physical activity or acute psychological stress, as indicated by the absence of concomitant group differences in sAA, which is predictive of SNS activity (see Granger et al., 2007 and Rohleder & Nater, 2009, for reviews). Finally, the enhanced basal HPA-axis activity could not be attributed to current depression, use of psychotropic medication or smoking. Based on these findings, it seems justified to conclude that our patients with PNES showed basal diurnal hypercortisolism.

There were no significant group differences in Cortisol Awakening Response (CAR), but in line with previous findings (Tunca et al., 1996) our patients with PNES seemed to show somewhat increased post-DST cortisol. Tunca et al. (1996) did not, however, control for psychotropic medication and smoking and when we adjusted for these factors our post-DST effects disappeared.

Our second aim was to test whether the increased HPA-axis activity would be particularly pronounced in traumatized patients with PNES. Based on previous findings of increased threat vigilance in PNES patients who reported sexual trauma (Bakvis et al., 2009a) we predicted to find higher cortisol levels in PNES patients with self-reported sexual trauma compared to patients without self-reported sexual trauma. Interestingly, the PNES patients in the present study reported significantly more sexual trauma compared to the HCs, but no group differences were found in the reports of emotional and physical trauma history, which may be interpreted as supporting the critical role of sexual trauma in PNES. Cortisol analyses indeed specified that, whereas both PNES patients with and without a history of sexual trauma showed increased basal cortisol levels compared to HCs, these findings were more pronounced for the traumatized patients (p = .067). This result is in line with previous findings in patients with depression showing increased cortisol levels only in traumatized depressed patients compared nontraumatized depressed patients (Heim et al., 2000b).

Relating our findings of increased HPA-axis activity in patients with PNES to other relevant stress-related disorders suggests that PNES show little overlap with Post Traumatic Stress Disorder (PTSD)[2]. In a systematic review and meta-analysis on basal cortisol levels in adult patients with PTSD, Meewisse et al. (2007) reported *lower* basal afternoon cortisol in female PTSD patients, particular in those patients reporting sexual or physical trauma. In addition cortisol hyper-suppression following DST (for a review see de Kloet, 2006) and increased nor-adrenergic activity (e.g. Southwick et al., 1999; Yehuda et al., 2001) have been reported in most studies in patients with PTSD. Similar signs of hypocortisolism have also been observed in stress-related bodily disorders, such as burnout, chronic fatigue, fibromyalgia and chronic pelvic pain (see Heim et al., 2000a for a review). Our findings of *hyper*cortisolism in PNES patients may more resemble previous findings in patients with a primary dissociative disorder. Increased basal 24-h urine cortisol was found in 46 patients with Dissociative Identity Disorder (DID) compared to HCs (Simeon et al., 2007). Post-DST cortisol was also increased in these DID patients as well as in 9 patients with Depersonalization Disorder (Simeon et al.,

[2] In Table 3.1, PTSD is not mentioned in the list of current comorbid psychopathology, indicating that none of the PNES patients fulfilled PTSD diagnostic criteria.

2001). Based on this scarce evidence, one might hypothesize that the HPA-axis hyperactivity in PNES patients shares more overlap with dissociative disorder than with PTSD. This notion is in accordance with previous suggestions that PNES might share some common underlying mechanism with dissociative disorder (Kuyk et al., 1996; Roelofs et al., 2002; Kihlstrom, 2005.; Brown et al., 2007).

Some strengths and limitations of the present study should be considered. A strength of the present study is that all patients were diagnosed using the gold standard: an ictal video-EEG registration of a typical seizure in order to confirm the absence of epileptiform activity, making PNES diagnosis maximally reliable (e.g. Reuber & Elger, 2003). Another strong point of this study is that relevant factors affecting the HPA-axis, such as age, gender, menstrual cycle, contraceptives, smoking, current depression and psychotropic medication were controlled for. On the other hand, although our sample size (N =18) was comparable to other clinical studies, we only had a small subgroup of nondepressed, nonsmoking and unmedicated patients (N=6). Therefore, this study needs to be replicated in a larger sample of patients with PNES. This might also offer an opportunity to further differentiate subgroups of PNES patients with respect to seizure-type or with respect to early childhood versus adulthood trauma. To further specification, it would be interesting to also include a control group of traumatized healthy participants. Another limitation of the study is that interpersonal trauma rates were based on a self-report questionnaire and were not verified using independent sources.

In conclusion, the results of the present study imply that PNES are associated with basal hypercortisolism, which was particularly pronounced in traumatized patients with PNES. The increased basal cortisol levels were not related to current seizures and there were no concomitant group differences in SNS activity. It, therefore, seems unlikely that the increased HPA-axis activity was merely due to group differences in current physical or acute psychological stress factors, but rather posits a relevant neurobiological marker for this stress-related disorder.

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CHAPTER 4

Basal cortisol is positively correlated to threat vigilance in patients with Psychogenic Non Epileptic Seizures

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Abstract

Previous studies have provided evidence for a vigilant attentional bias toward threat stimuli and increased basal diurnal cortisol levels in patients with Psychogenic Non Epileptic Seizures (PNES). Because cortisol levels may be predictive for threat vigilance, we reanalyzed previous data on threat vigilance in 19 unmedicated patients with PNES and found a positive correlation between baseline cortisol levels and attentional bias scores for threat stimuli (r=.49, p=.035). There was no such relation in healthy matched controls (n=20) or in patients with epileptic seizures (n=17). These findings provide the first evidence linking an endocrine stress marker to increased threat sensitivity in PNES and support new integrated psychoneurobiological models of PNES.

Introduction

Although Psychogenic Non Epileptic Seizures (PNES) are related by definition to psychological stress factors (APA, 1994), little is known about the cognitive and biological stress-sensitivity of patients presenting with PNES. Several studies have indicated that patients with PNES report higher rates of psychological trauma, such as sexual abuse, compared with healthy controls or controls with epilepsy (see Roelofs and Spinhoven, 2007 for a review). In addition, patients with PNES report more avoidant coping behavior (Frances et al., 1999; Goldstein et al., 2000; Reuber et al., 2005) and increased fear sensitivity (Hixon et al., 2006). However, all these findings rely on self-reports and to our knowledge only one study has investigated whether PNES are associated with increased threat sensitivity using an objective threat processing (reaction time) task. Bakvis et al. (2009a) found increased threat vigilance, as indicated by an attentional bias for displays of angry faces in an emotional Stroop task, in PNES as compared with matched healthy controls (HCs). In addition, two studies have reported increased basal cortisol levels in patients with PNES (Tunca et al., 2000; Bakvis et al., 2010a), one of which indicated that the basal hypercortisolism was independent of current seizures (Bakvis et al., 2010a). Cortisol may enhance processing of angry faces (Van Peer et al., 2007; 2009) and, although these findings are suggestive of a relation between basal cortisol levels and threat vigilance in patients with PNES, no studies have directly tested this premise. We reanalyzed previous data on threat vigilance in 19 unmedicated patients with PNES and related the previously reported attentional bias (AB) scores for angry faces (Bakvis et al., 2009a) to newly analyzed baseline (pre-task) cortisol levels. In addition, we tested the specificity of eventual effects by investigating the same relationship in the HCs reported in Bakvis et al. (2009a) and in a new control group of 17 patients with epileptic seizures (ES). We predicted that the cortisol levels would be positively correlated to the enhanced AB scores for angry faces in patients with PNES.

Methods

Participants

Nineteen patients with PNES and 20 HCs from the Bakvis et al. (2009a) study were included in the study. Patients with PNES, who were being treated at SEIN, Epilepsy Institute in the Netherlands, were recruited by the attending neurologists. The main inclusion criteria were (1) diagnosis of PNES based on an ictal video-EEG recording of a typical seizure and (2) no current use of medication (see Table 4.1 for demographics, seizure characteristics and menstrual cycle information and see Bakvis et al. (2009a) for detailed inclusion criteria). In addition, 17 patients with ES without suspicion of (a history of) comorbid PNES based on EEG recording (with or without additional neuroimaging data), medical history, seizure semiology and antiepileptic drug (AED) treatment experience, who were being treated at SEIN, were recruited by their neurologist. Sixteen patients with ES had localization related epilepsy (11 temporal lobe epilepsy (TLE), three frontal lobe epilepsy, two uncertain) and one patient had primary generalized epilepsy. AED treatment included monotherapy (n=15) with carbamazepine (n=9) or valproic acid (n=6) and polytherapy (n=1) with carbamazepine and clobazam. One patient was not on AED treatment.

Table 4.1: Group Characteristics.

Variable	HCs (N=20)	PNES (N=19)	ES (N=17)	Statistics
Age (SD) in years	22.1 (4.2)	27.6 (7.3)	42.4 (12.9)	$F(2,56)=26.6$, $p<.001$
Number of women	18	15	11	$X^2(2) = 3.5$, $p=.17$
using contraceptives[1]	10	6	1	$X^2(2) = 6.1$, $p<.05$
luteal phase[2]	8	7	4	X^2 (2) =0.48, $p=.79$
Mean age (SD) at Onset, years		21.1 (7.9)	20.7 (15.1)	$F(1,34)=0.01$, $p=.93$
Disease duration (SD), years		6.5 (7.4)	21.7 (15.7)	$F(1,34)=14.23$, $p<.01$

HCs, healthy control group; PNES, psychogenic non epileptic seizure group; ES, epileptic seizure group; [1]use of contraceptive was unknown in on PNES patient; [2]menstruation cycle was indeterminable in two patients with PNES and one HC; X^2, chi square.

All participants were instructed to minimize physical exercise during the hour preceding the experiment and to avoid large meals, coffee, drinks with low pH and cigarettes, because these variables can affect cortisol

levels. All participants had normal or corrected-to-normal vision. The study was approved by the local ethics committee and all participants provided written informed consent and received financial compensation for participation.

Measures

The emotional Stroop Task

The preconscious attentional processing of happy and angry faces was assessed using a masked pictorial emotional Stroop task (Van Honk et al., 1998). Facial stimuli of 10 different individuals (5 males, 5 females) were taken from Ekman and Friesen's Pictures of Facial Affect (Ekman and Friesen, 1976), each displaying a neutral, a happy and an angry expression. The facial stimuli were presented for 14 ms. Immediately after the stimulus presentation the pictures were replaced by a masking stimulus. The masking stimuli consisted of randomly cut, reassembled and rephotographed pictures of faces. At each trial, the stimulus and mask were presented in the same color (red, green or blue) and participants were instructed to vocalize this color as fast and accurately as possible. On vocal response initiation (timing of which was registered by means of voice-key registration: reaction time (RT) in ms), the presentation of the masking stimulus was terminated. After a random intertrial interval (2-4 s) new trials started with a 750 ms lasting fixation point. A total of 30 happy, 30 angry and 30 neutral faces were presented in a random order with the restriction that the same color was never repeated more than twice consecutively. The attentional bias (AB) score for angry faces was based on correct responses only, and calculated by subtracting the mean individual RTs for neutral face trials from the individual mean RTs for angry face trials.

Cortisol

Baseline cortisol was analyzed from saliva, sampled approximately 40 minutes before task administration using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Saliva 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), as described elsewhere (Van Aken et al., 2003).

Statistical analyses

Group differences in AB scores were analyzed using statistical analyses of variance (ANOVA) and subsequent LSD planned comparisons were calculated to further detail group differences. Correlations between baseline cortisol and AB scores were calculated using Pearsons correlations. Given the strong directedness of the hypotheses for the AB scores, group differences in AB scores were tested one-tailed, the other analyses were tested two-tailed (alpha 0.05). Effect sizes of significant results are reported using the Partial Eta Squared (η^2). Because groups differed with respect to age (see Table 4.1), we controlled for age by subsequently adding it as a covariate into the Group ANOVA for the AB scores. Because groups differed with respect to use of contraceptives by women (see Table 4.1), we controlled for this variable in case of significant effects involving cortisol (using partial correlations).

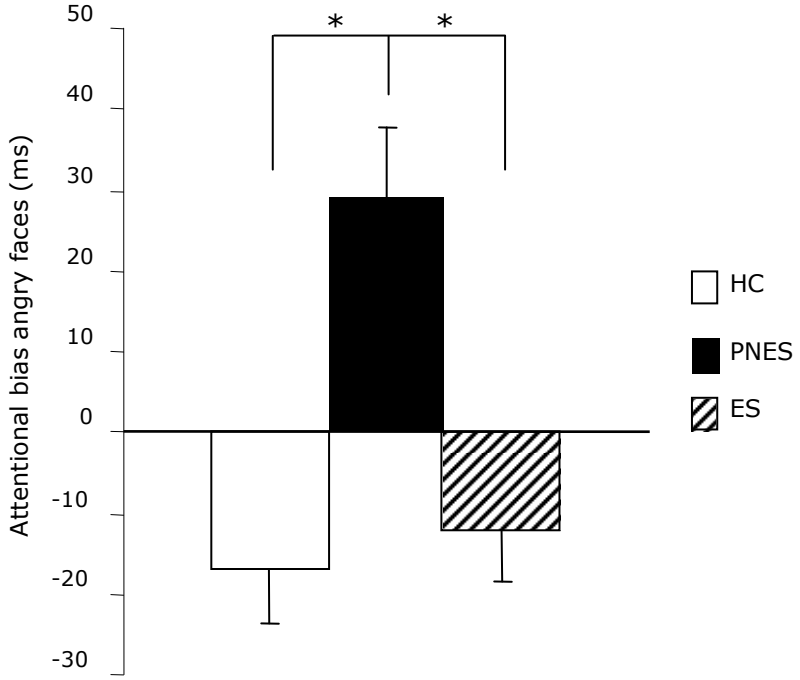

Figure 4.1. Attentional bias (AB) scores for angry faces (Reaction Time (RT) angry face trials - RT neutral face trials) for healthy controls (HCs), patients with psychogenic non epileptic seizures (PNES), and patients with epileptic seizures (ES); * $p \leq .05$.

Results

One-way ANOVA for the AB scores for angry faces, with Group (HCs, PNES, ES) as between-subject factor, indicated significant group differences ($F(2,56) = 2.85$, $p=0.033$, one tailed; $\eta^2=.097$, see Figure 4.1). This effect remained when controlling for age (age added as a covariate to the analysis: $F(3,56)=2.80$, $p=0.035$, $\eta^2=.097$). LSD planned comparisons indicated significant differences for patients with PNES versus those with ES ($p=0.032$) and versus HCs ($p=0.016$), but not for patients with ES versus HCs ($p=0.42$). Groups did not differ with respect to their baseline cortisol levels (HCs: M=6.7; SD=2.80; PNES: M=6.9; SD=2.96; ES: M=5.7; SD=3.10; $F(2,55)=0.95$, $p=0.39$) but, as expected, within the PNES group we found a significant positive correlation between the AB score for angry faces and the baseline cortisol levels ($r=0.49$, $p=0.035$, see Figure 4.2). This effect remained when controlling for menstrual cycle (r=0.49, $p=0.039$) and use of contraceptives ($r=0.49$, $p=0.037$) by means of partial correlations. There was no such relation for the HCs *(*$r=-0.001$, $p=0.99$) or ES ($r=-0.07$, $p=0.84$) control group for angry faces, and there were no such relations for happy faces in all groups (all $p>0.64$). Finally, we tested whether the reported correlations between baseline cortisol levels and the AB for angry faces differed significantly between the PNES and control groups. 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. Comparison of the correlations for patients with PNES with those for ES controls revealed a significant difference, as indicated by a *Z*-score (for independent groups, see Clark-Carter, 1997) of 1.64 ($p=0.05$) and the PNES-HCs comparison showed a trend towards significance, with $Z = 1.52$ ($p=0.064$).

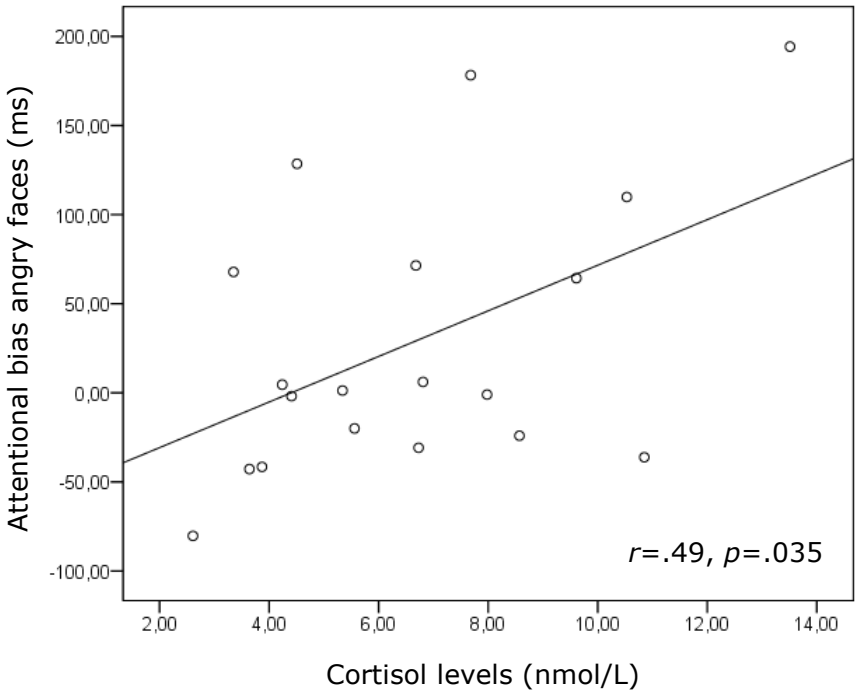

Figure 4.2. Correlation between pre-task cortisol levels and the attentional bias (AB) scores for angry faces in patients with psychogenic non epileptic seizures (PNES).

Discussion

This study showed that baseline (pretask) cortisol levels were positively correlated to threat vigilance in 19 unmedicated patients with PNES. These effects remained when controlling for use of contraceptives and menstrual cycle. The effects were specific for PNES and were absent for healthy individuals and patients with ES, respectively. The relationship between baseline cortisol and threat vigilance in patients with PNES in our study is relevant in the light of recent reports of increased basal cortisol levels observed in patients with PNES (Tunca et al., 2000; Bakvis et al., 2010a) and may contribute to our insight in possible stress-factors implicated in the increased threat vigilance in PNES. According to cognitive theories of medically unexplained symptoms (MUS: Brown, 2004) and more recent integrated psychoneurobiological theories of MUS (Roelofs and Spinhoven, 2007) increased activity in neurobiological stress systems and increased attention to threat make part of a state of hypervigilance that, in turn, may play a crucial role in

the presence of MUS as well as dissociative symptoms (Bakvis et al., 2009a; 2010a). In addition, increased threat vigilance on a masked emotional Stroop task (Hermans et al., 2006), as well as hypercortisolism (Simeon et al., 2007) have been reported for patients with a primary diagnosis of dissociative disorder as well. Taken together, these and previous findings in PNES show great overlap with previous findings in patients with a dissociative disorder. Although the findings need to be replicated, preferably in larger patient samples, the present results provide the first evidence of a direct relationship between the biological stress marker cortisol and cognitive threat sensitivity in PNES and provide a starting point, as well as preliminary support for integrated psychoneurobiological theories for this complex disorder (Roelofs and Spinhoven, 2007). If replicated, these findings together with evidence for increased basal cortisol levels in PNES (Bakvis et al., 2010a), may help to fine tune psychological as well as pharmacological interventions for PNES (LaFrance et al., 2006).

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CHAPTER 5

The effect of stress-induction on working memory in patients with Psychogenic Non Epileptic Seizures

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Abstract

Although Psychogenic Non Epileptic Seizures (PNES) are considered a stress-induced paroxysmal disintegration of cognitive functions, it remains unknown whether stress indeed impairs cognitive integrative functions, such as working memory (WM), in patients with PNES. A N-back task with emotional distracters (angry, happy and neutral faces) was administered at baseline and after stress-induction (Cold Pressor Test) to 19 patients with PNES and 20 matched healthy controls. At baseline, patients displayed increased WM interference for the facial distracters. After stress-induction, group differences generalized to the no-distracter condition. Within patients, high cortisol stress-responses were associated with larger stress-induced WM impairments in the no-distracter condition. These findings demonstrate that patients' cognitive integrative functions are impaired by social distracters and stress-induction. Moreover, the stress and cortisol related generalization of the relative WM impairments offers a promising experimental model for the characteristic paroxysmal disintegration of attentional and mnemonic functions in patients with PNES associated with stress.

Introduction

Psychogenic Non Epileptic Seizures (PNES) are paroxysmal, involuntary behavioral patterns that mimic epileptic events but lack ictal epileptiform activity in the brain and for which no organic cause can be identified. PNES are characterized by a sudden and time-limited alteration of consciousness and are associated with a disturbance in controlling cognitive, emotional and/or behavioral functions (e.g. Kuyk et al., 1999). This paroxysmal disintegration of attentional and mnemonic functions is thought to be associated with stress factors (WHO, 1993; APA, 1994; for reviews see Roelofs & Spinhoven, 2007; Reuber, 2009). Results of a few recent studies suggest that PNES are associated with increased stress sensitivity, as evidenced by increased cognitive threat vigilance (Bakvis et al., 2009a) and increased activity of biological stress systems (Tunca et al., 1996; 2000; Bakvis et al 2009a; 2010a). The ways in which threat and stress-induction interfere with working memory (WM), a profound cognitive integrative function that may be relevant to this disorder, remain unclear. This study was therefore designed to test the effects of threat and stress-induction on WM functions in patients with PNES.

We recently investigated attentional processing of masked threat stimuli in patients with PNES (Bakvis et al., 2009a). People with PNES have high interpersonal psychotrauma rates (for reviews see e.g., Fiszman et al., 2004; Sharpe and Faye, 2006; Roelofs & Spinhoven, 2007), and so pictures of angry facial expressions are considered relevant threat stimuli for them. We administered an emotional Stroop task in which angry, happy and neutral facial expressions were presented subliminally and backwardly masked. Compared to healthy control participants, patients with PNES displayed heightened interference for the masked angry faces specifically, indicating attentional hypervigilance for social threat cues at a preconscious level. Other studies showed increased activity of biological stress systems such as the Hypothalamus-Pituitary-Adrenal (HPA)-axis with cortisol as its end-product. Increased cortisol levels in patients with PNES were reported not only following a seizure (Tunca et al, 2000) but also temporally independent of seizure occurrence (Bakvis et al., 2010a). Patients with PNES also showed elevated cortisol levels associated with a delayed recovery of the HPA-

axis after dexamethasone administration in one study (Tunca et al., 1996) but not in another (Bakvis et al., 2010a). Finally, we found indications of decreased heart rate variability in patients with PNES (Bakvis et al., 2009a), also taken as an indication of hyperarousal and hypervigilance (see e.g. Thayer & Brosschot, 2005 for a review). Together these findings are suggestive of increased cognitive and biological stress-sensitivity in patients with PNES. These results may support the notion that, under circumstances of stress, patients with PNES show impairments in cognitive integrative functions (Bendefeldt et al., 1976; WHO, 1993) but such a relationship has not yet been tested directly.

One of the most crucial integrative cognitive functions needed for almost every voluntary action is working memory (WM). WM is a limited capacity system serving to maintain relevant information in short term memory and to suppress irrelevant information (Baddeley, 1996). On the basis of our previous research we hypothesized that social threat cues, such as angry facial expressions, as well as stress-induction, may significantly interfere with WM performance in patients with PNES. Threat interference with WM performance can be reliably tested using a N-back task with emotional distracters (Ladouceur et al., 2005). This task requires participants to monitor sequences of letters in various cognitive loads and to ignore the distracter pictures. We used several social (emotional) distracter pictures (pictures of angry, happy and neutral facial expressions) to test whether WM performance in patients with PNES would be more negatively affected by social threat distracter pictures compared to healthy controls (HCs). Second, because PNES are considered as a paroxysmal disintegration of attentional and mnemonic functions associated with stress, we tested whether stress-induction would result in a generalization of WM impairment to all distracter pictures in patients with PNES. The emotional WM task was therefore administered before and directly after stress-induction. WM performance is particularly sensitive to cortisol (e.g. Lupien et al., 1999; Elzinga & Roelofs, 2005, Oei et al., 2009), so we tested whether stress-induced cortisol was associated with the hypothesized generalization of WM impairment in patients with PNES. Stress was induced using the Cold Pressor Test (CPT). This physiological stress procedure consists of immersion of the nondominant hand in ice water and is known for its activating effect on both the Sympathetic Nervous System (SNS) and

the HPA-axis (e.g. Lovallo, 1975; Zimmer et al, 2003; Andreano & Cahill, 2006; Schoofs et al., 2009).

Methods

Participants

Patients with PNES who had been admitted to a tertiary epilepsy centre were recruited by the attending neurologists. Inclusion criteria were: (1) diagnosis of PNES based on an ictal video-EEG recording of a typical seizure, (2) PNES characterized by complete or partial loss of consciousness (specified as an ictal diminished or loss of adequate responsiveness, or postictal memory impairments of the ictal event), (3) the occurrence of at least two seizures in the year prior to the experiment, (4) no history of concomitant epileptic seizures, (5) no comorbid neurological disease diagnosis, (6) no diagnosis of endocrine disorder(s), and (7) signed informed consent.

The healthy control group was recruited through advertisements in local newspapers. Inclusion criteria were: (1) no psychiatric diagnosis, (2) no medical disease diagnosis, (3) no use of medication, and (4) signed informed consent.

Measures

Emotional N-back task (e-N-back)

WM performance was investigated at baseline and following the physiological stress-induction by the counterbalanced administration of 2 different versions of the emotional N-back (e-N-back) task. The e-N-back task is a modified version (based on e.g., Ladouceur et al., 2005) of the N-back WM task described by Cohen et al. (1994). The original N-back task consisted of visually presenting a pseudorandom sequence of letters and asking participants to respond to a prespecified letter. It included memory conditions whereby the load on WM varied as a function of the number of letters skipped for a target match. The N-back adapted for the present study included three workload conditions; 0-back, 2-back and 3-back. In the 0-back condition, participants monitored a sequence of letters for any occurrence of a single pre-specified letter. In the 2-back and 3-back condition participants observed a sequence of letters and responded by pressing the 'target'

button whenever the current letter was identical to the letter presented 2 and 3 trials back, respectively. Participants were instructed to respond by pressing the nontarget button if the presented letter did not meet the 'target' criterion. The target/nontarget buttons were counterbalanced for left/right between participants.

The e-N-back task consisted of superimposing the original N-back task onto one of four distracters (i.e. no picture, and pictures of neutral, happy and angry faces). Models were selected from Karolinska Directed Emotional Faces stimulus set (Lundqvist et al., 1998; Goeleven et al., 2008). In this way, the task consisted of three workload conditions (0-, 2- and 3-back) and four distracter conditions (no distracter and neutral, happy, and angry faces) leading to a total of 12 randomly assigned conditions. Each condition contained one block of 16 trials. Each trial consisted of three subsequent components; first, the inter-event-interval consisting of a black screen (500 ms), second, the presentation of the letter (e.g. P) superimposed on the distracter (500 ms) and third, an asterisk (*) superimposed on the distracter (2500 ms). Each block of 16 trials was preceded by six practice trials. Within each facial background block 16 different models displaying the same emotion were presented once and the same models were used for every facial background block. The male/female ratio was counterbalanced within each block.

WM performance was operationalized using error rates (errors of omission and errors of commission) and reaction times (RTs). It is inherent to the 3-back condition that the first 3 trials are nontarget trials. To keep the different workload conditions as comparable as possible, the first 3 trials for all 12 blocks contained nontarget trials that were excluded from analyses, leaving 6 nontarget and 7 target trials within each block, and a total of 156 (72 nontarget and 84 target) trials in the e-N-back task.

Effort and compliance

WM performance is directly influenced by the amount of effort employed by the participants, so we determined participants` efforts by administrating the Amsterdam Short Term Memory test (Amsterdamse Korte Termijn Geheugen taak; AKTG; Schmand et al., 1998). This relatively simple task requires participants to read five neutral words aloud and then perform a single distracting arithmetic task. Subsequently, five more words are given, three of which were previously

presented. The participants are instructed to name the three words that were previously presented. The score is determined by the number of words named correctly. No points are awarded for the arithmetic tasks, as these serve as distracter items. When employing large amounts of effort, all participants without evident cognitive disorders should be able to complete this relatively simple task, with few errors, in approximately 10 minutes. An error rate of $\geq$ 5 indicates task underachievement (Schmand et al., 1998). As a result, participants with an AKTG score of $\geq$ 5 were excluded from subsequent analyses.

Furthermore, to ensure that potential group differences in WM performance were not due to poor effort or lack of compliance, response patterns during the WM task were analysed to check for irregularities. Response patterns consisting of pressing both the target and the nontarget button within one trial; or pressing either the target or the nontarget button consistently within one block were considered as signs of noncompliance.

Anxiety and Depression

The Symptom Check List Revised (SCL-90-R) is a self-report questionnaire that evaluates a broad range of psychological problems and symptoms of psychopathology (Derogatis, 1977; Arrindell and Ettema, 2003). SCL-90-R consists of 90 items and each item inquires about recent physical and psychological complaints that can be scored on a 5-point scale ranging from 0='not at all' to 4='very much'. We administered the Anxiety and Depression subscales in the present study. The Anxiety subscale consists of 10 items and subsumes a set of symptoms usually associated clinically with high manifest anxiety (i.e. restlessness, nervousness, tension). The Depression subscale consists of 16 items and reflects a broad range of signs and symptoms of the clinical depressive syndromes (i.e. dysphoric affect, withdrawal of interest in life activities, loss of vital energy).

Cold Pressor Test (CPT)

After the baseline administration of the e-N-back task, participants were requested to immerse their nondominant hand up to the wrist in an ice-cold water bath (0-4 °C) for as long as possible up to a maximum of 3 minutes. This procedure was repeated 3 times at standardized but unpredictable intervals (1 to 4 minutes). The CPT or plunge test is known to elicit a robust stress-response and to activate the SNS and

HPA-axis simultaneously (e.g. Lovallo, 1975; Zimmer et al, 2003; Andreano & Cahill, 2006; Schoofs et al., 2009).

Physiological and subjective measures

To test the effectiveness of the stress-induction, several physiological and subjective stress measures were registered. All physiological and subjective stress-measures were obtained at nine assessment points over approximately a 145-minute period with five baseline assessment points prior to the stressor, and four assessment points after the stressor. Because of the natural fluctuations of cortisol during the day, all assessments were performed at the same time of day between 1.15 pm and 4.00 pm.

Hypothalamus pituitary adrenal (HPA)-axis. Saliva samples for cortisol assessments were obtained using Salivette collection devices with a cotton roll (Sarstedt, Rommelsdorf, Germany). Saliva sampling (in contrast to blood sampling) is a stress-free noninvasive way to measure cortisol (Kirschbaum et al., 1993; Kirschbaum & Hellhammer, 1994). Saliva samples were stored at –20 ºC until assayed at a suitable laboratory (http://biopsychologie.tu-dresden.de). Cortisol concentrations in saliva were measured using a commercially available chemiluminescence-immuno-assay kit with high sensitivity (IBL, Hamburg, Germany). Inter- and intra-assay coefficients of variation were below 10%.

Sympathetic nervous system (SNS). Systolic (SBP) and diastolic blood pressure (DBP) were measured in the nondominant arm using an automatic electronic digital blood pressure monitor, the Omron R5-I, initiated manually. This device met the validation criteria of international guidelines for both systolic and diastolic blood pressure (for more information see Omboni et al., 2007).

Subjective anxiety and pain. During saliva sampling, participants were asked to register their subjective experience of anxiety and pain on a visual analogue scale (VAS, 0-100).

Procedure

All physiological and subjective stress-measures were obtained at nine assessment points over approximately a 145-minute period, at

respectively -75, -60, -40, -25, 0 (rest), +15, +35 (stress), +50 and +70 (recovery) minutes with reference to the start of the stressor. All assessments were performed between 1.15 pm and 4.00 pm. See also Figure 5.1.

Candidate participants were invited for an initial session in which they were informed about the specifics of the experiment. With respect to the stress-induction procedure, it was explained that stress would be induced by means of a physiological stress procedure, but no further details were provided, in order to prevent possible anticipation effects. On the test day, participants arrived 2 hours prior to the first physiological assessment and over two hours before the cognitive tasks were administered. All participants were previously instructed to minimize physical exercise during the hour preceding the experiment and to avoid large meals, coffee, drinks with low pH or cigarettes, because these variables can affect cortisol levels. After participants had provided informed consent, they were each screened for DSM-IV axis I disorders (APA, 1994), using a semi-structured diagnostic interview (assessed using the MINI: Mini-International Neuropsychiatric Interview; Sheenan et al 1998; Van Vliet & De Beurs, 2007). No later than 30 minutes after arrival, participants had a light lunch (sandwiches and soft drinks). Half an hour later the DSM-IV screening was continued (if necessary), and then the SCL-90-R was administered. At 1.15 pm the first physiological assessment took place (-75 minutes with reference to the start of the stressor, see also Figure 5.1), followed by a 15 minute relaxation period prior to the second physiological assessment (-60 minutes). Directly after the second physiological assessment, the e-N-back task was administered for the first time, followed by the third physiological assessment (-40 minutes). Two other cognitive tasks were administered, the details of which will be published elsewhere (Bakvis et al., resubmitted). After the fifth physiological assessment (0 minutes), the CPT was administered. The sixth physiological assessment (+15 minutes) took place immediately following the CPT, and preceded the second administration of the e-N-back task, followed by the seventh physiological assessment (+35 minutes). The AKTG was administered at the end of the experiment when cortisol returned back to baseline levels. For a schematic overview of the entire experiment, see Figure 5.1.

The protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC). All participants received financial compensation for participating in the experiment.

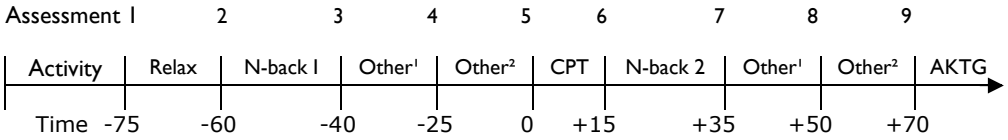

Figure 5.1. A schematic overview of the experiment. Physiological and subjective stress-measures were obtained at nine assessment points, at respectively -75, -60, -40, -25, 0 (rest), +15, +35 (stress), +50, +70 minutes with reference to the start of the stressor. CPT, Cold Pressor Test; AKTG, Amsterdamse Korte Termijn Geheugen taak; ¹ Approach-Avoidance Task; ² Sternberg Task.

Statistical analyses

WM performance was operationalized as the percentage errors of total given answers, and reaction times (RTs). To normalize distributions, RTs were subjected to natural log transformation before analyses. Possible group differences in WM performance, physiological, and subjective stress measures were analyzed using repeated measures analyses of variance (ANOVA rm), and subsequent planned comparisons (post hoc Least Significant Difference, LSD contrasts) were calculated to further detail differences. In case of significant group effects in WM, reanalysis without patients who were on psychotropic medication was performed. To investigate specificity of possible group findings and to statistically control for the amount of variance explained by anxiety and depressive symptoms, we subsequently added SCL-90-R Anxiety and Depression subscale scores as covariates in the WM analysis. Correlations between cortisol and WM scores were calculated using Spearman Rho correlations. All analyses were tested two-tailed (alpha 0.05).

Results

Participants

Effort and compliance. Of the 25 patients with PNES and the 23 HCs who participated in the current study, six patients and three HCs were post hoc excluded from analyses. The first patient 'failed' the effort

test with an error rate exceeding 5, a score indicative of task underachievement. Five patients and two HCs were excluded from analyses based on the response pattern analysis of the e-N-back task; one patient and one HC pushed both the target and the nontarget response button for the greater part of the trials, four patients and one HC pushed only one button (either the target or the nontarget) in $\geq$ 2 conditions. One HC was excluded because the majority of the saliva samples did not contain sufficient saliva for cortisol analyses.

Demographics. The remaining 19 patients (15 females) had a mean age of 35.3 (*SD*=11.4) years. Demographic data, menstrual cycle, use of contraceptives, use of psychotropic medication, smoking status, and seizure characteristics are provided in Table 5.1. The remaining 20 HCs (15 females) had a mean age of 31.2 (*SD*=12.7) years. Patients and HCs did not differ significantly with respect to age, gender, education, use of contraceptives, menstrual cycle and smoking status (see Table 5.1). As expected, more patients used psychotropic medication. Patients had higher scores than HCs on both the Anxiety and Depression subscales of SCL-90-R (see Table 5.1 for further details).

Table 5.1. Demographic and clinical characteristics for 19 patients with PNES and 20 healthy controls (HCs).

Variable	Patients (N = 19)	Controls (N = 20)	Statistics
Mean age (SD) in years	35.3(11.4)	31.2(12.7)	$F(1,37)=1.14$, ns
Number of women	15	15	$X^2(1) = .09$, ns
using contraceptives	8	5	$X^2(2) = 1.29$, ns
follicular phase[1]	3	6	$X^2(2) = 1.22$, ns
Education			$X^2(1) = 3.31$, ns
primary/secondary	14	9	
higher	5	11	
Smokers	9	9	$X^2(1) = .62$, ns
Taking psychotropic medication	5	0	$X^2(1) = 6.04$, *
Mean score (SD) SCL-90-R Depression subscale	31.2 (13.80)	18.75 (3.73)	$F(1,37)=15.04$, **
Mean score (SD) SCL-90-R Anxiety subscale	19.1 (8.10)	11.55 (1.79)	$F(1,37)=16.34$, **
Mean seizure frequency per 4 weeks[2](SD)	10.5 (22.46)	-	

[1]menstruation cycle was indeterminable in one patient and one control participant; [2] one patient did not report seizure frequency; ** p <.001; * p <.05; ns p is not significant.

Manipulation checks: Stress-induction

CPT duration. There were no group differences in the total mean time (in minutes) that participants kept their hand in the ice-water [PNES 3.6 (*SD*=3.5); HCs 5.5 (*SD*=3.5); ($F(1,37)=2.70$, $p=.104$)].

Cortisol. A two-way ANOVA rm for the salivary Cortisol levels with Time (nine assessment points) as within-subjects factor and Group (patients, HCs) as between-subjects factor showed a main effect for Time ($F(8,30)=9.35$, $p<.001$) and a non-significant trend for Group ($F(1,37)=3.01$, $p=.091$). There was no significant Time X Group interaction ($F(8,30)=1.42$, $p=.230$). For both groups, cortisol levels were increased following the CPT (average cortisol level for assessments 6-7) compared to baseline (average cortisol level for assessments 1-5), see Figure 5.2 ($F(1,37)=4.15$, $p<.049$).

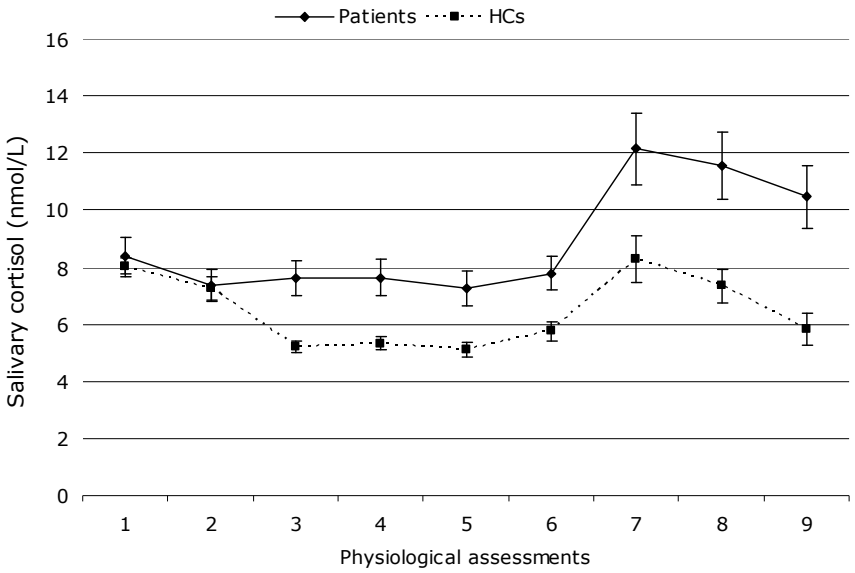

Figure 5.2. Mean (SEM) cortisol levels for patients and HCs for the nine physiological assessment points.

Blood pressure. Separate two-way ANOVAs rm for SBP and DBP with Time as within-subjects factor and Group as between-subjects factor showed main effects for Time [SBP ($F(8,30)=2.76$, $p=.020$), DBP ($F(8,30)=8.27$, $p<.001$)] but no significant effects involving Group (all $p\geq.311$). In both groups, blood pressure was increased following CPT

(average BP level for assessments 6-7) compared to baseline (average BP level for assessments 1-5) [SBP (F(1,37)= 6.11, p=.018); DBP (F(1,37)=5.48, p=.025)].

Subjective anxiety and pain. Separate two-way ANOVAs rm for subjective anxiety and pain with Time as within-subjects factor and Group as between-subjects factor showed main effects for Time [anxiety (F(8,30)=2.89, p=.017); pain (F(8,30)=5.21, p<.001)]. Again there were no significant effects involving Group (all $p\geq$.172). Both groups experienced more pain following CPT (average pain level for assessments 6-7) compared to baseline (average pain level for assessments 1-5) (F(1,37)= 32.85, p<.001). Neither group reported increased anxiety following CPT compared to baseline (F(1,37)= 2.56, p=.118).

Together these findings indicate that physiological stress-induction by the CPT was successful. A statistical trend towards a group difference was found only for cortisol; patients showed slightly higher cortisol levels throughout the experiment.

Emotional working memory performance

Error rates. To test possible group differences in error rates, we conducted a four-way ANOVA rm with Phase (baseline, stress), Distracter (no-distracter, neutral, happy and angry faces) and Workload (0-, 2- and 3-back) as within-subject factors and Group (patients, HCs) as between-subject factor. A significant main effect for Group (F(1,37)=6.96, p=.012)[3] indicated that patients made overall more errors than HCs (see Figure 5.3). Most crucially, there was a significant interaction for Phase X Distracter X Group (F(3,35)=3.58, p=.023)[4]. Adding both SCL-90-R Anxiety and Depressive symptoms subscales as covariates into this analysis, did not alter these effects [(Group (F(1,35)=6.96, p=.004; Phase X Distracter X Group F(3,33)=3.69, p=.021) suggesting that these effects were not related to group differences in anxiety and depressive symptoms. Figure 5.3 illustrates group differences at baseline for the social distracter conditions and

[3] This main effect remained significant when patients on psychotropic medication (N=5) were excluded from analysis (F(1,32)=5.37, p=.027).

[4] This three-way interaction effect remained significant when patients on psychotropic medication (N=5) were excluded from analysis (F(3,30)=3.39, p=.031).

generalization of the group differences to the no-distracter condition following stress. This observation was supported by subsequent analyses. Post hoc *F* tests for each distracter condition separately, demonstrated no significant main effect for Group ($F(1,37)=2.82$, $p=.102$) but a significant Group X Phase effect for the no-distracter condition ($F(1,37)=6.06$, $p=.019$) indicating that both groups did not differ on the no-distracter condition at baseline ($F(1,37)=.65$, $p=.800$), but patients made significantly more errors than HCs on the no-distracter condition following CPT ($F(1,37)=6.10$, $p=.018$), because of a relative improvement following CPT compared to baseline in HCs ($F(1,19)=5.71$, $p=.027$) and an absence of such an improvement in patients ($F(1,18)=1.40$, $p=.253$). Main effects for Group were present for both angry and neutral faces [($F(1,37)=8.05$, $p=.007$) and ($F(1,37)=8.04$, $p=.007$) respectively] and a statistical trend in the same direction for happy faces [($F(1,37)=3.78$, $p=.060$)]. The Phase X Group interactions were not significant for any of the facial distracter conditions (all $p \geq .189$). In line with these results, additional post hoc *F* tests indicated that the Distracter X Group interaction was only significant at baseline ($F(3,35)=3.02$, $p=.043$), not following CPT ($F(3,35)=1.69$, $p=.188$). At baseline, HCs did not display a main effect for Distracter ($F(3,17)=.60$, $p=.626$), but the patients did ($F(3,16)=6.72$, $p=.004$). Post hoc LSD analyses indicated that within the patient group at baseline, the no-distracter condition differed significantly from all facial distracter conditions (all $p \leq .003$), whereas the facial distracter conditions did not differ mutually (all $p \geq .398$). There were no other significant interactions for Group present (all $p > .323$).

To summarize: at baseline, patients displayed more WM interference than HCs for the facial distracter conditions but not for the no-distracter condition. Following stress-induction, group differences in WM impairment generalized to all conditions including the no-distracter condition.

Reaction Times. A four-way ANOVA rm for RTs with Phase (baseline, stress), Distracter (no, neutral, happy and angry faces) and Workload (0-, 2- and 3-back) as within-subject factors and Group (patients, HCs) as between-subject factor showed no significant (interaction) effects involving Group (all $p \geq .100$).

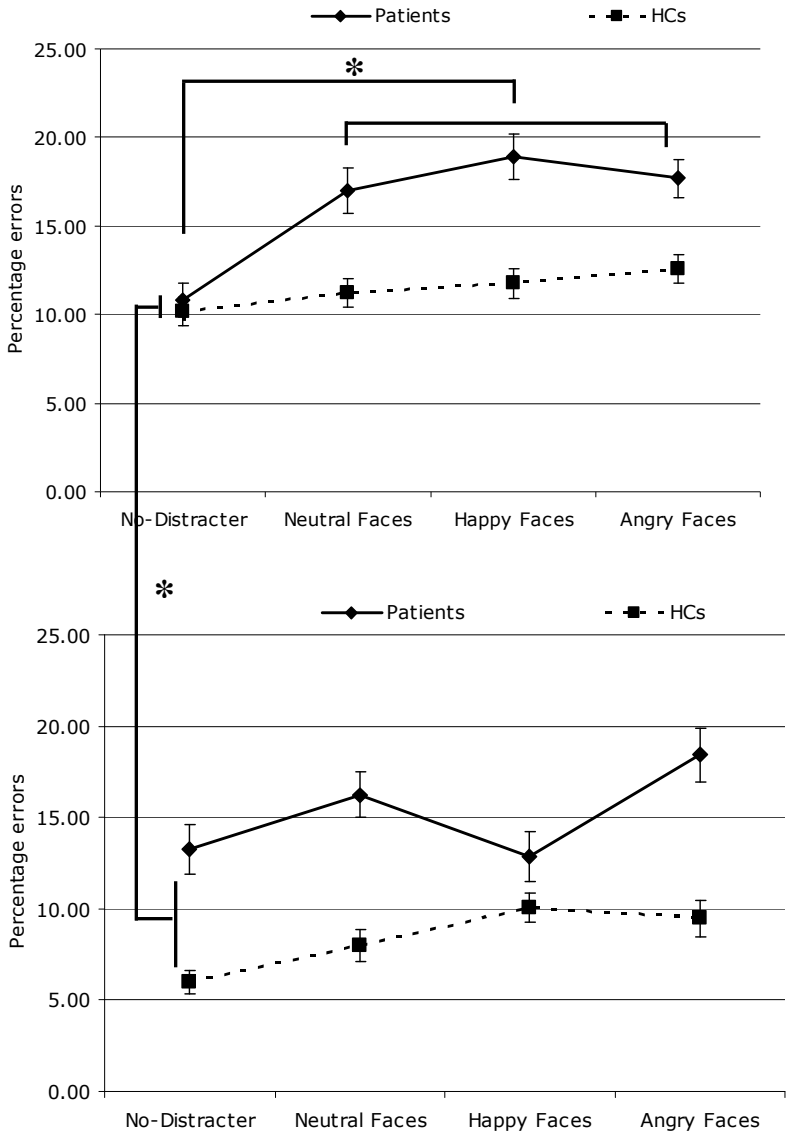

Figure 5.3. Emotional N-back (e-N-back) mean percentage (SEM) error rates per distracter condition for patients and healthy controls (HCs) during baseline (upper panel) and after stress-induction (lower panel). At baseline, patients displayed more WM interference than HCs for the facial distracter conditions but not for the no-distracter condition. Following stress-induction, group differences in WM impairment generalized to all conditions including the no-distracter condition.

Error rates and cortisol

To investigate whether the stress-induced effect for the no-distracter condition was associated with stress-induced cortisol responses, we conducted a correlational analysis for the difference scores in WM (error rates following CPT - error rates baseline) and percentage cortisol stress-response ((cortisol +20 min - cortisol 0 min)/cortisol 0 min * 100). Results showed a significant positive effect for the patient group (Spearmen Rho R=.46, p=.046), but not for the HCs (R=.05, p=.818). This finding indicates that patients with high cortisol stress-responses also had the most pronounced stress-induced WM declines for the no-distracter condition.

Discussion

The aim of this study was to test the effects of social threat and physiological stress-induction on WM performance in patients with PNES. Three major findings emerged: First, the presence of social distracters resulted in an impairment of patients' WM performance, both at baseline and after stress-induction. Second, although patients' general (no-distracter) WM performance was unimpaired at baseline, a significant group difference in this no-distracter condition emerged after stress-induction. Whereas HCs improved after stress-induction, the patients did not show such an improvement. Third, patients with high cortisol stress-responses had larger stress-induced WM impairments in the no-distracter condition. Below we will detail these findings and discuss their implications.

As expected, the current results showed more WM interference by social (facial) threat stimuli in PNES patients than in HCs. Interestingly and contrary to our expectations, this was not only the case for angry face distracters, but also for happy and neutral face distracters. Compared to HCs, patients made more errors on the e-N-back task when the, to be remembered, letters were positioned on distracting social background stimuli. RT analysis showed no significant group effects, making it unlikely that patients' increased error rates are due to shorter response latencies. The results were also unrelated to group differences in anxiety and depressive symptoms. The present finding of increased interference of social distracters, irrespective of working load condition, may indicate

that the social distracters are potent stimuli that affect cognitive performance in patients with PNES regardless of cognitive load. The current findings are in line with a recent fMRI study in patients with positive motor conversion symptoms, showing increased amygdala activity compared to HCs in response to viewing both positive and negative facial expressions using an incidental affective task (Voon et al., 2010). They furthermore extend previous findings of increased cognitive interference by social threat stimuli in patients with PNES (Bakvis et al., 2009a), although the previous study indicated increased attentional interference specific for *angry* faces in patients with PNES. The latter discrepancy might be explained by several methodological differences, for example in the Stroop study faces were presented subliminally and backwardly masked, whereas in the present study the faces were presented for 3 seconds. Also, the tasks differed in complexity. The Stroop task requires simple color-naming of the masks that follow the subliminally presented faces, whereas the e-N-back task is a complex task consuming the limited resources of the WM system.

For patients with PNES, WM performance without distracters was unimpaired at baseline, suggesting that there is no general WM deficit on the N-back task in patients with PNES. The presence of any social distracter, however, caused sufficient interference to lead to significantly impaired WM function in the patient group. Interestingly, after stress-induction the general WM functions of HCs improved, but such improvement did not occur in patients with PNES, resulting in a generalization of group differences in WM impairment, that is, patients made more errors on the whole compared with HCs, irrespective of whether a background distracter was presented or not. The improvement of general WM functions in HCs following (mild) stress-induction is in line with previous studies reporting improved WM performance in HCs in a mild naturalistic stress context (Lewis et al., 2008). Also, Lupien et al. (1999) demonstrated that mild doses of exogenous cortisol administration had beneficial effects on WM performance, similar to the improvement of WM performance in HCs following stress. However, with varying the levels of administered cortisol, Lupien et al. were able to demonstrate an inverted U shaped curve for the effect of cortisol on WM performance, where relatively low as well as relatively high levels of exogenous cortisol appeared to have negative effects on WM performance (Lupien et al., 1999). On the basis

of these results, it is not unlikely that our patients with PNES, with their slightly increased cortisol levels already at baseline, have not benefited from an addition cortisol boost due to stress-induction, because they (unlike HCs) may have found themselves already on the downward slope of the inverted U shaped curve.

Another interesting finding was that patients had slightly elevated cortisol levels throughout the experiment (p=0.9) and that, in patients with PNES, higher cortisol stress-responses were associated with larger stress-induced WM impairments in the no-distracter condition. These results resemble earlier findings of a positive association between pre-task cortisol and impaired cognitive performance in patients with PNES (Bakvis et al., 2009b), indicating that patients' cognitive impairments may, at least partly, be associated with increased activity of neurobiological stress systems. The current finding of a statistical trend towards increased cortisol throughout the experiment in patients with PNES is in accordance with previous studies showing increased cortisol at baseline (Tunca et al., 2000; Bakvis et al., 2010a).

Strengths and limitations

Before discussing the implications of the current findings, some strengths and limitations of the present study should be considered. All patients were diagnosed using the gold standard; an ictal video-EEG registration of a typical seizure in order to confirm the absence of epileptiform activity, making PNES diagnosis maximally reliable (Reuber and Elger, 2003). Based on a recent discussion suggesting that poor neurological functioning in patients with PNES might be associated with poor effort during task performance (Cragar et al., 2006; Drane et al., 2006; Locke et al., 2006; Dodrill, 2008), we thoroughly investigated indications of poor effort and compliance by administrating a malingering task and by WM task response patterns analyses. As a result we excluded six patients, who did not pass our malingering test or who showed signs of poor compliance based on the response pattern analyses. We also excluded two HCs based on these criteria, illustrating the importance of studying effort and compliance in cognitive experiments in general. Another strength of the present study is that participating HCs were similar to patients with respect to several relevant factors such as age, gender, menstrual cycle, contraceptives, smoking and educational level. Other patient characteristics such as

increased depressive and anxiety symptoms, and psychotropic medication were statistically controlled for. It therefore seems reasonable to conclude that the present findings are specifically associated with PNES, and not to (random) factors varying between both groups. A limitation of the current study is the use of social background distracters only. Consequently, it is impossible to determine whether WM performance in patients with PNES was impaired by the social disposition of the distracters or by the presence of distracters per se. Future studies should therefore also include nonsocial distracters. Second, additional investigations should address the question regarding which stage of WM is most affected in patients with PNES. WM involves the temporary storage, maintenance, and manipulation of information and involves several dissociable components: future studies should utilize an experimental paradigm isolating the different stages of WM performance. Mechanisms for the storage of information have been associated with posterior brain structures, whereas mechanisms for manipulation of information, or executive control, have been associated with anterior brain structures (D'Esposito and Postle, 2002). It is also unclear whether patients' increased social distracter interference is associated with increased limbic processing of the distracters or with decreased frontal inhibition of irrelevant information, or both. Therefore, it would be interesting for future studies to test patients' cognitive impairments and their neural correlates using brain imaging techniques. Finally, there is a clear need to explore the association of patients' cognitive impairments with their symptomatology, for example by assessing patients before and after (successful) treatment.

To summarize, this is the first study to demonstrate that, compared with HCs, WM performance in patients with PNES is impaired by social distracting stimuli. Not only threatening distracters, but also neutral and positive social distracters interfered with patients' WM performance. Stress-induction resulted in a generalization of these group differences to the no-distracter condition. Interestingly, those patients who had the largest stress-induced cortisol responses also showed the largest stress-induced impairments in general (no-distracter) WM performance. Together, these findings indicate that patients with PNES have problems inhibiting irrelevant social-emotional stimuli. In addition, generalization of WM deficits following stress may mimic the paroxysmal disintegration of attentional and mnemonic functions in patients with PNES associated with stress.

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CHAPTER 6

Automatic avoidance tendencies in patients with Psychogenic Non Epileptic Seizures

The content of this chapter is resubmitted to Seizure;
Bakvis P., Spinhoven P., Zitman F.G., & Roelofs K.

Abstract

Introduction. Psychogenic Non Epileptic Seizures (PNES) have been theorized to reflect a learned pattern of avoidant behavior to deal with stressors. Although such observation may be relevant for our understanding of the etiology of PNES, evidence for this theory is largely build on self-report investigations and no studies have systematically tested actual *avoidance behavior* in patients with PNES. In this study, we tested automatic threat avoidance tendencies in relation to stress and cortisol levels in patients with PNES and healthy controls (HCs).

Methods. The approach-avoidance (AA) task was administered to 12 patients with PNES and 20 matched HCs at baseline and following stress-induction using the Cold Pressor Test (CPT). The AA task requires participants to evaluate the emotional valence of pictures of angry and happy faces by making arm movements (arm flexion or extension) that are either affect-congruent (avoid-angry; approach-happy) or affect-incongruent (approach-angry; avoid-happy) with their intuitive action tendencies. Saliva cortisol was measured throughout the experiment.

Results. Patients, but not HCs, showed increased approach-avoidance congruency-effects for angry faces on the AA task at baseline, with relatively slower approach of angry faces, which was overall associated with basal pre-task cortisol. This congruency-effect disappeared after the CPT.

Discussion. The present findings provide an objective confirmation of previous suggestions from self-report studies indicating that PNES patients show relatively increased avoidance tendencies to social threat cues. The registering of threat *avoidance behavior* may prove to be a clinically valuable contribution to evaluate psychological treatment effectiveness and perhaps even PNES prognosis.

Introduction

Avoidance behavior is hypothesized to be an important precipitating and perpetuating factor for Psychogenic Non Epileptic Seizures (PNES-Reuber, 2009). It has been suggested that PNES are a learned pattern of avoidant behavior to deal with stressors (Ramani et al., 1980). The ictal state of altered awareness associated with PNES is also said to act as an avoidance response to protect the individual from experiencing stressful events or from memories of those events (Goldstein et al. 2000). Findings from self-report investigations suggest that patients with PNES experience their lives as more stressful and use more maladaptive, escape and avoidance oriented coping strategies, i.e. behavioral efforts to avoid conflicts or stress, compared to healthy controls HCs (Frances et al., 1999; Goldstein et al. 2000). Increased dissociative tendencies have also been found in patients with PNES (Goldstein et al. 2000; 2006) and this has been considered to protect individuals from unacceptable psychological stress factors and may therefore be considered as an avoidant coping strategy (Gross, 1983).

Most evidence so far comes from self-report measures, but there are also a few neurobiological and experimental findings supporting the increased stress- and threat sensitivity in patients with PNES. For example, PNES patients showed increased basal cortisol levels (Tunca et al., 2000; Bakvis et al., 2010a), and high cortisol levels have been widely associated with increased avoidance tendencies (Van Honk et al., 1998; Roelofs et al., 2005a; 2009a; Van Peer et al., 2007; 2009). A recent experimental study has also shown that PNES patients demonstrate an increased attentional bias for angry faces, but not for happy faces (Bakvis et al., 2009a) and this was related to basal cortisol levels Bakvis et al., 2009b). These results of increased biological and cognitive stress-sensitivity in patients with PNES may be consistent with the commonly self-reported avoidant strategies to cope with stressors, but actual threat avoidance *behavior*, hypothesized to precipitate and perpetuate the disorder, has not yet been objectively tested in patients with PNES.

A systematic and objective method to study human avoidance behavior to social threat stimuli is provided by the social approach-avoidance (AA) task (Rotteveel and Phaf, 2004). This reaction time task requires participants to evaluate the emotional valence of pictures of

angry and happy faces by making arm movements (arm flexion or extension) that are either congruent or incongruent with their intuitive action tendencies (see Rotteveel and Phaf (2004) for a photograph of the task-set up). Affect-congruent movements involve arm-extension (avoidance) in response to a negative stimulus (angry face) and arm-flexion (approach) in response to a positive stimulus (happy face). Affect-incongruent movements involve reversed mappings (i.e. approach-negative and avoid-positive stimuli). With this paradigm a congruency-effect is typically found, indicating faster responses for affect-congruent arm movements than for affect-incongruent arm movements (see also Roelofs et al., 2005a; Solarz, 1960; Chen and Bargh, 1999). This task is sensitive to anxiety (Heuer et al., 2007; Roelofs et al., 2009a; 2010) and cortisol (Van Peer et al., 2007; 2009): anxiety and increased cortisol levels have consistently been found to be associated with increased congruency-effects for angry faces, indicative of social avoidance tendencies.

In the present study we tested whether PNES patients showed increased threat avoidance tendencies by administrating the AA task to patients with PNES and healthy control participants. Since PNES patients typically report to display increased avoidant behavior in stressful circumstances, the AA task was administered before and after stress-induction, allowing us to evaluate whether social threat avoidance behavior in patients was even more pronounced following stress-induction. Stress was induced using the Cold Pressor Test (CPT). This physiological stress procedure consists of immersing the nondominant hand in ice water, which is known for its activating effect on both the Sympathetic Nervous System (SNS) and the HPA-axis, resulting in increased cortisol levels (e.g. Lovallo, 1975; Zimmer et al, 2003; Andreano & Cahill, 2006; Schoofs et al., 2009). We investigated whether increased cortisol was associated with the hypothesized increased social threat avoidance behavior in patients with PNES (Bakvis et al., 2009a; 2009b; 2010a).

Methods

Participants

Patients with PNES, who had been admitted to a tertiary epilepsy centre, were consecutively recruited by attending neurologists between

March 2008 and August 2009. Inclusion criteria were: (1) diagnosis of PNES based on an ictal video-EEG recording of a typical seizure, (2) PNES characterized by complete or partial loss of consciousness (specified as an ictal diminished or loss of adequate responsiveness or post-ictal memory impairments of the ictal event), (3) the occurrence of at least two seizures in the year prior to the study, (4) no history of concomitant epileptic seizures, (5) no comorbid neurological disease diagnosis, (6) no diagnosis of endocrine disorder(s), (7) age between 18 and 65 years, and (8) signed informed consent.

The healthy control group was recruited through advertisements in local newspapers. Inclusion criteria were: (1) no psychiatric diagnosis, (2) no medical disease diagnosis, (3) no use of medication, (4) age between 18 and 65 years, and (5) signed informed consent.

Measures

Approach-Avoidance (AA) task

In this affect-evaluation task (Rotteveel and Phaf, 2004) participants responded to visually presented pictures of emotional facial expressions by making arm movements toward (arm flexion or approach) or away from (arm extension or avoid) their own body. Eighty grayscale photographs displaying angry or happy facial expressions served as stimuli (Ekman and Friessen, 1976; Matsumoto and Ekman, 1988; Lundqvist et al., 1998). Both the happy and angry expressions were taken from the same models (total of 40 models; 50% female). The stimuli were subdivided into four fixed series (A1-A2-B1-B2) each with 10 happy and 10 angry expressions from different models. The approach and avoidance responses were given by means of three one-button boxes that were fixed to a vertical stand. 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 in the middle loosely with the back of the right hand as long as no response was given. The response buttons were positioned above the home-button for the flexion arm movement and below the home-button for the extension arm movement. This allowed participants simply to 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 (happy or angry) and to respond as fast and accurately as

possible to the stimuli by releasing the home button and pressing one of the response buttons. After this, they returned their hand to the home button. All participants 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 instructions 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 instruction before and after stress-induction was counterbalanced across participants. Each instruction block was followed by 12 practice trials containing pictures not included in the actual AA task. The start of an individual trial was indicated by the appearance of a central fixation point (100 ms). After an interval of 300 ms the stimulus was presented for 100 ms followed by an inter-trial-interval of 1500 ms. This task provides two behavioral measures, i.e. median reaction times (RT: time between stimulus onset and response) and error rates (percentage incorrect responses).

Anxiety and Depression

The Symptom Check List Revised (SCL-90-R), a self-report questionnaire, was used to assess levels of anxiety and depression (Derogatis, 1977; Arrindell and Ettema, 2003). The Anxiety subscale consists of 10 items, the Depression subscale of 16 items. Each item inquires about recent physical and psychological complaints that can be scored on a 5-point scale ranging form 'not at all' to 'very much'.

Cold Pressor Test (CPT)

Participants were instructed to immerse their nondominant hand up to the wrist in an ice-cold water bath (0-4 $^{\circ}$C) for as long as possible (maximum of 3 minutes). This procedure was repeated 3 times at standardized but unpredictable intervals (1 to 4 minutes). The CPT or plunge test is known to elicit a robust stress-response and simultaneously to activate the SNS and HPA-axis (e.g. Lovallo, 1975; Zimmer et al, 2003; Andreano & Cahill, 2006; Schoofs et al., 2009).

Cortisol

In order to test the effectiveness of the stress-induction, saliva samples for cortisol assessments were registered at 9 assessment points over approximately a 145-minute period, divided in a rest, stress and a recovery phase, at respectively: rest: -75, -60, -40, -25, 0; stress: +15, +35; and recovery: +50 and +70 (recovery) minutes with reference to the start of the stressor. All assessments were performed between 1.15 pm and 4.00 pm.

Saliva samples were obtained using Salivette collection devices with a cotton roll (Sarstedt, Rommelsdorf, Germany). Saliva sampling (in contrast to blood sampling) is a stress-free noninvasive way to measure cortisol (Kirschbaum et al., 1993; Kirschbaum & Hellhammer, 1994). Saliva samples were stored at –20 ºC until assayed at a suitable laboratory (http://biopsychologie.tu-dresden.de). Cortisol concentrations in saliva were measured using a commercially available chemiluminescence-immuno-assay kit with high sensitivity (IBL, Hamburg, Germany). Inter- and intra-assay coefficients of variation were below 10%.

Procedure

Candidate participants were invited for an initial informative session in which they were informed about the specifics of the experiment. With regards to the stress-induction procedure, they were told that stress would be induced by means of a physiological stress procedure, without providing further details in order to prevent possible anticipation effects. On the test day, participants arrived two hours prior to the first cortisol assessment took place and over two hours before the cognitive tasks were administered. All participants were previously instructed to minimize physical exercise during the hour preceding the experiment and to avoid large meals, coffee, drinks with low pH and cigarettes, because these variables can affect cortisol levels. After participants provided informed consent, all participants were administered a semi-structured diagnostic interview, to screen for DSM-IV axis I disorders (APA, 1994; assessed using the MINI: Mini-International Neuropsychiatric Interview Sheenan et al 1998; Van Vliet & De Beurs, 2007). No later then 30 minutes after arrival, participants had a light lunch (sandwiches and soft drinks). Half an hour later the DSM-IV screening was continued (if necessary), subsequently the SCL-90-R was administered. At 1.15 pm the first cortisol assessment took

place (-75 min. with reference to the onset of the stressor see Figure 6.1), followed by a 15 minute relaxation period prior to the second cortisol assessment (-60 min). Directly following the second assessment, a cognitive task was administered of which the details will be published elsewhere (Bakvis et al., 2010b), followed by the third cortisol assessment (-40 min). The AA task was administered following the third and prior to the fourth assessment, see also Figure 6.1. After the fifth cortisol assessment (0 min), the CPT was introduced and administered. The second administration of the AA task followed the seventh cortisol assessment (+35 min).

The protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC). All participants received financial compensation for participating in the experiment.

Statistical analyses

Reaction time (RT) outliers were filtered with a <150 and > 1000-msec cut-off. For each participant, the median of the remaining RTs (99%) for the correct responses was calculated per cell (defined by Condition [baseline, post-CPT], Valence [angry, happy], and Movement [approach, avoidance]). Group differences in approach-avoidance tendencies to angry faces on the AA task were analyzed (Roelofs et al., 2009a) using repeated measures analyses of variance (ANOVA rm) with Arm movement (approach-avoidance) and Phase (baseline, post-CPT) as within-subjects factors and Group (PNES, HCs) as between-subject factor. Subsequent planned comparisons (post hoc Least Significant Difference, LSD contrasts) were calculated to detail differences further. Effect sizes of significant results are reported with the Partial Eta Squared (η^2). In case of significant group effects in social threat avoidance behavior, we tested whether the effects were due to medication by reanalyzing data excluding patients who were on psychotropic medication. To investigate specificity of possible group findings, we statistically controlled for Anxiety and Depressive symptoms by subsequently adding SCL-90-R Anxiety and Depression subscale scores as covariates in the analysis. To assess correlations between approach-avoidance tendencies for angry faces and cortisol, we performed Pearson correlation coefficient between individual AA congruency-effects for angry faces (RT incongruent angry face trials -

RT congruent angry face trials) and pre-AA task cortisol levels. All analyses were tested two-tailed (alpha 0.05).

Results

Table 6.1. Demographic variables and group characteristics for patients with PNES and HCs.

Variable	Patients (N = 12)	Controls (N = 20)[1]	Statistics
Mean age (SD) [years]	36.8(12.9)	31.9(12.7)	$F(1,30)=1.12$, $p=.299$
Gender (*n*: male/female)	4M/8F	5M/15F	$X^2(1) = .26$, $p=.612$
Women (*n*: yes/no):			
using contraceptives	5Y/3N	6Y/9N	$X^2(2) =.54$, $p=.762$
follicular phase[2]	3Y/5N	6Y/9N	$X^2(2) =.23$, $p=.988$
Smokers (*n*: yes/no)	8Y/4N	7Y/13N	$X^2(1) =3.02$, $p=.082$
Mean score (SD) SCL-90 depression	37.4 (19.1)	19.4 (4.08)	$F(1,30)=16.93$, $p<.001$**
Mean score (SD) SCL-90 anxiety	23.3 (11.9)	11.9 (2.03)	$F(1,30)=17.93$, $p<.001$**
Seizure characteristics			
mean age (SD) at onset [years]	33.1 (13.0)	-	
mean disease duration (SD) [years]	3.8 (3.0)	-	
mean frequency per 4 weeks (SD)	10 (19.1)	-	
Medication (*n*)[3]			
current psychotropic medication	4	-	
SSRI	4		
benzodiazepine	2		
current AEDs	0	-	
previous AEDs	5	-	
levetiracetam	2		
carbamazepine	4		
valproate	2		
Current DSM IV-axis I disorders (*n*)[4]	10	-	
mood disorder	3		
anxiety disorder			
agoraphobia	5		
panic disorder	2		
general anxiety disorder	2		
post traumatic stress disorder	4		
obsessive compulsive disorder	1		
somatoform disorder			
chronic pain	1		

[1] the majority of saliva samples for one HC did not contain sufficient saliva for cortisol analysis; [2] menstruation cycle was indeterminable in one patient and two control participants; [3] because some patients used more than one AED or psychotropic medication, the sum of *n* exceeds the total *n;* [4] because patients often met more than one DSM IV axis I criteria, the sum of *n* exceeds the total *n;* ** p <.001; * p <.05.

Participants

Participants were 12 patients (mean age 36.8 (*SD*=12.9) years; 8 female) and 20 HCs (mean age 31.9 (*SD*=12.7) years; 15 female)[5]. Demographic data, menstrual cycle, use of contraceptives, use of psychotropic medication, smoking status, and seizure characteristics are provided in Table 6.1. Eleven patients had been or were being treated according to the psychological treatment program described in Kuyk et al. (2008). The last patient received psychiatric treatment in his home region. Patients and HCs did not differ significantly with respect to age, gender, education, use of contraceptives, menstrual cycle and smoking status (see Table 6.1). As expected, more patients used psychotropic medication and had higher scores than HCs on both the Anxiety and Depression subscales of SCL-90-R (see Table 6.1 for further details).

Manipulation check: Cortisol response

A two-way ANOVA-rm for the salivary Cortisol levels with Time (9 assessment points) as within-subjects factor and Group (patients, HCs) as between-subjects factor showed main effects for Time ($F(8,22)=7.94$, $p<.001$, $\eta^2=.743$) and Group ($F(1,29)=16.02$, $p<.001$, $\eta^2=.356$). There was no significant Time X Group interaction ($F(8,22)=.75$, $p=.649$). As shown in Figure 6.1, the patient group had elevated cortisol levels compared with HCs throughout the experiment. In addition, for both groups, the pre-task cortisol levels were significantly lower in the baseline condition (assessment 3) than after stress-induction (assessment 7 ($F(1,29)=6.50$, $p<.016$, $\eta^2=.183$)), indicating that stress-induction using the CPT was successful for both groups. Thus, although patients with PNES showed increased cortisol levels at baseline, stress-induction led to comparable increases in cortisol levels in each group.

[5] A total of 25 patients and 23 HCs participated in this experiment, but due to a technical problem, RTs were incompletely registered for 13 patients and 3 HCs, who were therefore excluded from subsequent analyses.

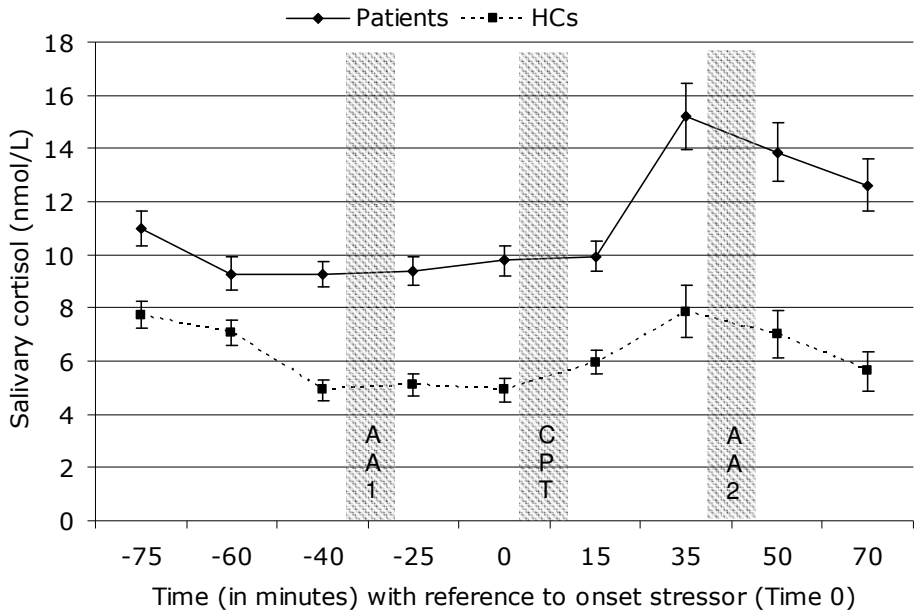

Figure 6.1. Cortisol levels (± SEM) for 12 patients and 20 HCs for the 9 physiological assessment points. AA1, first administration AA task; AA2, second administration AA task; CPT, Cold Pressor Test.

Approach-Avoidance (AA) task

RTs Angry faces. An ANOVA-rm for the RTs for angry faces with Phase (baseline, post-CPT) and Arm movement (approach, avoidance) as within-subject factor and Group (PNES, HCs) as between-subject factor showed no main effects for Group ($F(1,30)=1.32$, $p=.260$), Arm movement ($F(1,30)=.01$, $p=.915$) and Phase ($F(1,30)=.02$, $p=.881$). There was a non-significant trend towards a Group X Arm movement interaction ($F(1,30)=3.97$, $p=.056$). Most importantly, there was a Phase X Group X Arm movement interaction ($F(1,30)=6.84$, $p=.014$, $\eta^2=.186$). Post hoc *F* tests for each Phase separately indicated that the Group X Arm movement interaction was significant at baseline ($F(1,30)=8.13$, $p=.008$, $\eta^2=.213$) but not following stress ($F(1,30)=0.00$, $p=.984$). This significant effect at baseline remained significant when both SCL-90-R Anxiety and Depressive symptoms subscale scores were entered as covariates into the analysis (Group X Arm movement ($F(1,28)=6.73$, $p=.015$, $\eta^2=.194$), suggesting that this effect was not related to group differences in anxiety and depressive symptoms. This Group X Arm movement interaction also remained

significant at baseline ($F(1,26)=13.40$, $p=.001$, $\eta^2=.340$) when four patients who used psychotropic medication were excluded from this analysis, demonstrating that this effect could not be explained by medication use. Interestingly, as can be seen in Figure 6.2, only the patients displayed a significant effect for Arm movement at baseline ($F(1,11)=5.10$, $p=.045$, $\eta^2=.317$). Patients were slower in affect-incongruent (angry-approach) trials than in affect-congruent (angry-avoid) trials. HCs did not show such an effect for Arm movement ($F(1,19)=.72$, $p=.408$). Additionally, at baseline, patients responded significantly slower than HCs to the affect-incongruent (angry-approach) trials ($F(1,30)=4.73$, $p=.038$, $\eta^2=.136$) and this was not the case for the affect-congruent (angry-avoid) trials ($F(1,30)=.32$, $p=.577$).

RT Happy faces and error rates. The same ANOVA-rm for happy faces resulted in no significant effects involving Group (all $p>.273$, for a complete overview of RTs see Table 6.2). Similar analyses for the error rates resulted in no significant effects.

Thus, PNES patients showed increased approach-avoidance congruency-effects for angry faces on the AA task at baseline, with relatively slower approach of angry faces. This effect was significantly reduced (and disappeared) after the CPT.

Threat avoidance and baseline cortisol

To test whether approach-avoidance tendencies for angry faces at baseline were overall correlated with the pre-task cortisol levels, we calculated a Pearson correlation coefficient between the individual RT congruency-effects for angry faces (RT incongruent angry faces – RT congruent angry faces) and pre- task cortisol levels (both at baseline) and found a positive correlation ($r=.38$, $p=.034$[6]). As expected, participants with high basal pre-task cortisol showed increased delays for the incongruent angry face trials relative to the congruent trials.
When each group was tested separately, the correlations did not reach significance [patients ($r=.23$, $p=.482$); HCs ($r=.00$, $p=.100$)[7]].

[6] Repeating this analysis with the 3 basal pre-task cortisol measurements calculated with Area Under the Curve with respect to ground (AUCg; for more details see Pruessner et al., 2003, formula 2), resulted in a comparable outcome ($r=.37$, $p=.040$).

[7] Repeating this analysis with the 3 basal pre-task cortisol measurements calculated with AUCg resulted in comparable outcomes [patients ($r=.29$, $p=.359$); HCs ($r=.12$, $p=.637$)].

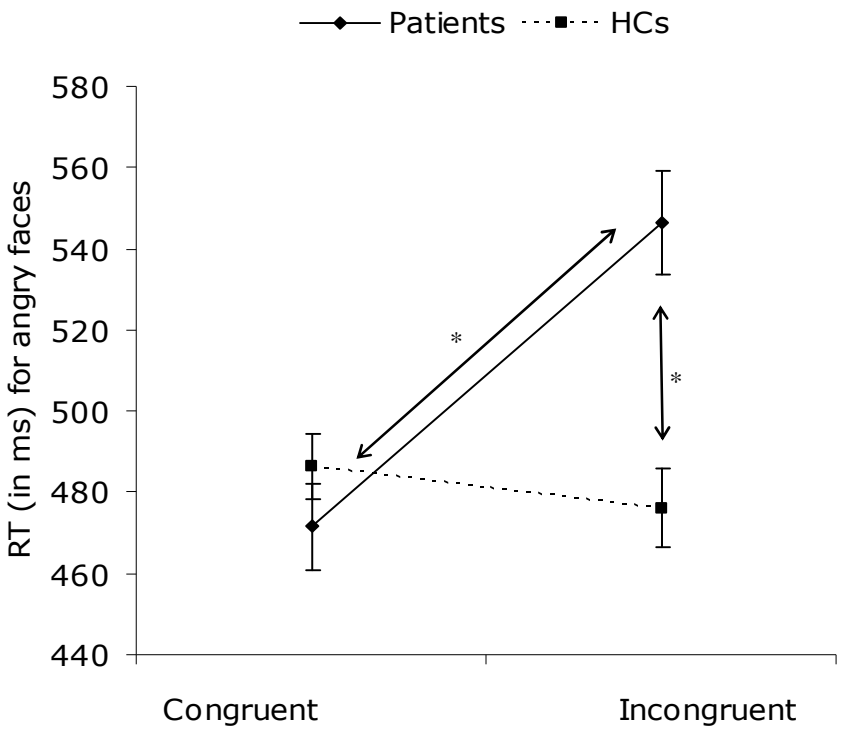

Figure 6.2. RTs (± SEM) for congruent and incongruent angry face trials for 12 patients and 20 HCs on the Approach-Avoidance (AA) task. Patients show increased AA congruency-effects for angry face responses with a relative slowing to approach compared to avoid angry face stimuli; * p<.05.

Table 6.2. Overview of RTs (± SEM) and % error rates separately for group, phase, arm movement and emotion.

Group	Phase	Arm movement	Emotion	RT	% Error
Patients (*n*=12)	Baseline	Congruent	Happy	457,54 (21,59)	13.3
			Angry	471,46 (20,94)	15.0
		Incongruent	Happy	518,58 (27,04)	12.5
			Angry	546,54 (25,59)	8.3
	CPT	Congruent	Happy	500,29 (25,19)	5.0
			Angry	525,46 (26,09)	5.8
		Incongruent	Happy	512,63 (26,36)	10.8
			Angry	495,08 (27,96)	4.2
HCs (*n*=20)	Baseline	Congruent	Happy	458,20 (16,73)	5.0
			Angry	486,40 (16,22)	11.5
		Incongruent	Happy	475,63 (20,94)	9.5
			Angry	476,18 (19,82)	7.5
	CPT	Congruent	Happy	465,65 (19,51)	9.5
			Angry	491,58 (20,21)	12.0
		Incongruent	Happy	477,20 (20,42)	7.5
			Angry	461,68 (21,66)	9.5

Discussion

The aim of this study was to investigate avoidance behavior in patients with PNES. Specifically, we aimed to test suggestions from previous self-report studies suggesting that PNES is associated with increased threat avoidance tendencies. Secondly, we tested whether social avoidance behavior was increased after stress-induction and whether it was related to cortisol levels. Three relevant findings emerged from this study. First, patients with PNES showed increased avoidance tendencies to social threat cues on the AA task at baseline. Secondly, the overall angry face congruency-effect was related to baseline cortisol levels. Thirdly, stress-induction did not further increase but rather decreased the angry face congruency-effect in patients with PNES. Below we will detail these findings and discuss their implications.

The finding of a relative preference to avoid rather than approach angry faces in patients with PNES may be interpreted as being in line with previous findings from self-report studies of increased avoidant coping in patients with PNES (Frances et al., 1999; Goldstein et al., 2000; 2006). The AA congruency-effect in patients with PNES was specific for angry faces, and did not occur for happy faces. Previous studies using approach-avoidance tasks showed increased avoidance tendencies to angry faces in anxious populations (Heuer et al., 2007; Roelofs et al., 2009a; 2010). When we statistically controlled for anxiety the congruency-effect for angry face responses in patients with PNES remained significant, indicating that these findings cannot be fully attributed to the patients' self-reported anxiety levels. The angry face congruency-effect in patients with PNES could also not be explained by other patient characteristics such as increased depressive symptoms, and use of psychotropic medication. As a result it seems justified to conclude that the finding of a relative delay in threat approach behavior may be a specific marker associated with PNES. The relative preference to avoid rather than to approach angry face cues observed in patients in this experimental set-up was mainly attributed to a relative slowing when patients had to make an approaching arm-movement to angry faces compared both to HCs and to their own angry-avoid trials. These results indicate that the behavior op patients with PNES is not affected by angry faces when their behavior is in accordance with their instinctive avoidant action tendency, but when they have to behave in a manner

incongruent to their instinctive avoidant action tendency in response to social threat stimuli, i.e. approach, behavioral interference occurs. Such reaction time cost is generally observed when an automatic motor response (avoidance of angry face) needs to be inhibited in favor of the selection of a rule driven motor response conflicting with this automatic action tendency (approach angry faces- Roelofs et al., 2009a). Recent fMRI studies using this task have shown that the left ventrolateral prefrontal cortex (vlPFC) plays a crucial role and is significantly recruited during these affect-incongruent response conditions (Roelofs et al., 2009b; Volman et al., in press). The fact that patients demonstrated altered approach-avoidance behavior in response to angry faces extends previous findings of an increased attentional bias for angry faces in patients with PNES (Bakvis et al., 2009a), now showing that angry faces not only draw more attention but also elicit relative inhibition of approach-related motor responses.

Previous investigations using self-report measures indicated that PNES patients report reliance on avoidance behavior particularly in stressful situations (Frances et al., 1999; Goldstein et al., 2000; 2006). Based on these results, we expected patients to display even more pronounced threat avoidance behavior following stress-induction. Contrary to our expectations, however, no behavioral group differences were present following the CPT. These results are in line with previous experimental findings in patients with PNES in which the attentional vigilance for angry faces at baseline was no longer present after stress-induction (Bakvis et al., 2009a). Possible explanations for this normalization following stress-induction may be associated with the after-math effects of the stress-induction procedure. The CPT stress paradigm includes a social evaluative component and the investigator who is present during the CPT, is also present during the second administration of the AA task. This may have resulted in a decreased significance of the emotional value of the angry faces during the second administration of the AA task.

Previous investigations have shown that behavioral responses to (social) threat are related to cortisol levels (Roelofs et al., 2005a; 2009a; Van Peer et al., 2007; 2009). In this study we confirmed these findings showing a positive association between pre-task baseline cortisol and the relative slowing in angry face approach behavior at baseline for both groups. When testing this association within both groups separately, a comparable positive, but non-significant, relation

was found in the patient group, which may become significant when tested in larger groups. We found no association between baseline cortisol and the angry face congruency-effect in the HCs.

Before discussing the implications of the current findings, some strengths and limitations of the present study should be considered. An important strength of this study is that all patients were diagnosed using the gold standard (see e.g. Reuber and Elger, 2003 for a review): an ictal video-EEG registration of a typical seizure in order to confirm the absence of epileptiform activity, making PNES diagnosis maximally reliable. Another strength of the present study, besides statistically controlling for patient characteristics such as depressive symptoms, anxiety and medication use, is that participating HCs were comparable to patients based on several relevant factors such as age, gender, menstrual cycle, use of contraceptives, and smoking, minimizing the effect of random factors on the present results. The most obvious limitation of the present study is the relative small patient group size, and its associated limited statistical power. We therefore emphasize that the present results need to be interpreted with caution and surely need replication. Future studies could investigate the social threat approach-avoidance tendencies in patients with PNES using larger groups of patients to further unravel the specific effects of cortisol on their threat approach and avoidance tendencies. Based on findings of Selkirk et al. (2008) differentiating PNES patients reporting sexual abuse from PNES patients not reporting a sexual abuse history, it would be interesting for future studies to investigate the effect of sexual abuse on threat avoidance tendencies in patients with PNES. This is especially relevant since previous studies demonstrated that patients with PNES, who report a sexual trauma, displayed increased attentional interference by angry faces and demonstrated elevated cortisol levels compared to PNES patients without a sexual trauma report (Bakvis et al., 2009a; 2010a). The additional use of brain imaging techniques would provide an opportunity to investigate whether altered vlPFC activity is associated with increased difficulty to inhibit automatic threat avoidance tendencies in patients with PNES, or whether group differences in social threat avoidance behavior are rather associated with increased limbic activity associated with social threat processing, or both. Also, because patients with PNES report using increased avoidant coping strategies in stressful situations, it would be interesting to study threat avoidance behavior in patients with PNES using cortisol administration (see e.g. Van Peer et

al., 2007). Cortisol administrations prevents possible attentional confounds induced by a real life stress-induction, which may have been associated with a stress-induction protocol used in the present and an earlier study (Bakvis et al., 2009a). Finally, because avoidance behavior is considered as an important precipitating and perpetuating factor for PNES (Ramani et al., 1980; Goldstein et al., 2000; Reuber, 2009) adequate use of coping strategies and fear avoidance are focuses in most therapies used for PNES [e.g. (Kuyk et al., 2008; Goldstein et al., 2010]. It may be worth investigating whether changes in patients' self-report coping strategies and avoidance behavior are confirmed by changes in automatic threat avoidance tendencies after successful treatment. In addition, it would be clinically highly relevant to investigate whether (changes in) threat approach and avoidance behavior could serve as a predictor for PNES prognosis.

Conclusion

The present results suggest increased social threat *avoidance behavior* in patients with PNES at baseline, which was overall associated with basal pre-task cortisol. Positive emotional stimuli did not affect behavioral approach-avoidance responses in patients with PNES and their behavioral threat avoidance responses normalized after stress-induction. Because PNES are considered as avoidant behavior to cope with threatening and stressful situations (Ramani et al., 1980; Goldstein et al., 2000), the objective registering of social threat avoidance behavior may prove to be a clinical valuable contribution to evaluate psychological treatment effectiveness and perhaps even the prognosis of PNES.

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CHAPTER 7

Summary and Discussion

PNES are considered as a paroxysmal disintegration of cognitive functions associated with psychological stress factors. Self-report studies have found indications of increased stress sensitivity in patients with PNES, and psychological stress and trauma, as well as maladaptive avoidant behaviors to deal with threatening and stressful situations, have been acknowledged as important etiological factors in PNES (e.g. Reuber, 2009). The primary aim of the present thesis was to use an integrative approach of cognitive and neurobiological stress research to test the assumptions of increased cognitive and neurobiological stress sensitivity in patients with PNES. Secondly, we aimed to investigate how possible findings of increased cognitive and neurobiological stress sensitivity may influence a) important cognitive integrative functions, and b) avoidance behavior in patients with PNES.

The present chapter first provides an overview of the main findings reported in chapters 2-6, followed by a discussion of the results in relation to the previously formulated hypotheses and a subsequent integration of the present findings. Then, a discussion of the strengths and limitations of the studies described in chapters 2-6 will follow. Finally, this chapter concludes with suggestions for future research and possible implications of the present findings for clinical practice.

Overview of the main results

Chapter 2. PNES have long been considered as paroxysmal dissociative symptoms characterized by an alteration of attentional functions due to severe stress or trauma. Although interpersonal trauma is common in PNES, the proposed relation between trauma and attentional functions remains underexplored. We examined the attentional processing of social threat in PNES in relation to interpersonal trauma and acute psychological stress. Therefore, a masked emotional Stroop test, comparing color-naming latencies for backwardly masked angry, neutral and happy faces, was administered to 19 unmedicated patients with PNES and 20 matched healthy controls (HCs), at baseline and in a stress condition. Stress was induced by means of the Trier Social Stress Test (TSST) and physiological stress parameters, such as heart rate variability (HRV) and cortisol, were measured throughout the experiment. Results indicated that no group differences related to the acute stress-induction or cortisol were found. Compared to HCs, however, patients displayed a positive attentional

bias for masked angry faces at baseline, which was correlated to self-reported sexual trauma. Moreover, patients showed lower HRV at baseline and during recovery. These findings are suggestive of a state of hypervigilance in patients with PNES. The relation with self-reported trauma, moreover, offers the first evidence linking psychological risk factors to altered information processing in patients with PNES.

Chapter 3. Only a few studies have examined the associations with neurobiological stress systems, such as the Hypothalamus-Pituitary-Adrenal (HPA)-axis with its end-product cortisol. We tested several relevant parameters of HPA-axis functioning in PNES patients and related them to trauma history. Cortisol awakening curve, basal diurnal cortisol and negative cortisol feedback (using a 1 mg Dexamethasone-Suppression-Test) were examined in 18 PNES patients and 19 matched HCs using saliva cortisol sampling on two consecutive days at 19 time-points. Concomitant sympathetic nervous system (SNS) activity was assessed by analyzing saliva alpha-amylase (sAA). Patients with PNES showed significantly increased basal diurnal cortisol levels compared to HCs. This effect was mainly driven by patients reporting sexual trauma who showed a statistical trend towards higher cortisol levels as compared to patients without a sexual trauma report. Importantly, the increased basal diurnal cortisol levels in patients could not be explained by depressive symptoms, medication, smoking, or by current seizures or group differences in SNS activity. This is the first study showing that basal hypercortisolism in patients with PNES is independent from the acute occurrence of seizures. In addition, basal hypercortisolism was more pronounced in traumatized PNES patients as compared to nontraumatized PNES patients. These findings suggest that increased basal salivary cortisol levels form a neurobiological marker for PNES.

Chapter 4. Previous studies provided evidence for a vigilant attentional bias toward threat stimuli (Chapter 2) and increased basal diurnal cortisol levels (Chapter 3) in patients with PNES. Because cortisol levels may be predictive of threat vigilance, we reanalyzed previous data on threat vigilance in 19 unmedicated patients with PNES and found a positive correlation between baseline cortisol levels and attentional bias scores for threat stimuli. There was no such relation in the 20 matched HCs or in the 17 patients with epileptic seizures. These findings provide the first evidence linking an endocrine stress marker to increased threat sensitivity in patients with PNES and support new integrated psychoneurobiological models of PNES.

Chapter 5. Although PNES are considered as a stress-induced paroxysmal disintegration of cognitive functions, it remains unknown whether stress and stress-induced cortisol impairs important cognitive integrative functions needed for almost all voluntary planned action, such as working memory (WM), in patients with PNES. WM performance was tested using an N-back task with emotional distracters (photos of angry, happy and neutral faces), requiring participants to monitor sequences of letters in various cognitive loads and to ignore the distracters. This task was administered at baseline and after stress-induction, using the Cold Pressor Test (CPT), to 19 patients with PNES and 20 matched HCs. Saliva cortisol was measured throughout the experiment. Patients with PNES demonstrated a normal cortisol stress-response on top of their slightly elevated basal cortisol levels, therefore, cortisol levels were slightly increased in patients with PNES throughout the experiment. At baseline, patients displayed increased WM interference for the facial distracters. After stress-induction, group differences generalized to the no-distracter condition. Contrary to our expectations, we did not find a relation between WM performance and basal cortisol, but within patients, high cortisol stress-responses were associated with larger stress-induced WM impairments in the no-distracter condition.

These findings demonstrate that patients' cognitive integrative functions are impaired by social distracters. Moreover, the stress and cortisol related generalization of the relative WM impairments offers a promising experimental model for the characteristic paroxysmal disintegration of attentional and mnemonic functions in patients with PNES associated with stress.

Chapter 6. PNES have been theorized to reflect a learned pattern of avoidant behavior to deal with stressors. Although such observation may be relevant for our understanding of the etiology of PNES, evidence for this theory is largely build on self-report investigations and no studies have systematically tested actual avoidance tendencies in patients with PNES. In the same experiment as described in Chapter 5, we furthermore tested automatic threat avoidance tendencies in patients with PNES in relation to stress and cortisol levels. The approach-avoidance (AA) task was administered at baseline and following stress-induction using the CPT. The AA task requires participants to evaluate the emotional valence of pictures of angry and happy faces by making arm movements (arm flexion or extension) that are either affect-

congruent (avoid-angry; approach-happy) or affect-incongruent (approach-angry; avoid-happy) with their intuitive action tendencies. Due to technical problems, AA task data was only available for 12 patients with PNES and 20 matched HCs. Saliva cortisol was measured throughout the experiment. Contrary to HCs, patients showed increased approach-avoidance congruency-effects for angry faces on the AA task at baseline, with relatively slower approach of angry faces, which was overall associated with basal pre-task cortisol. This congruency-effect disappeared after the CPT. The present findings provide an objective confirmation of previous suggestions from self-report studies indicating that PNES patients show relatively increased avoidance tendencies to social threat cues.

Testing the hypotheses

As is described in Chapter 1, we aimed to test the following hypotheses: 1). Patients with PNES display increased cognitive threat sensitivity. 2). Patients with PNES display increased neurobiological stress sensitivity. 3). Patients' increased cognitive and neurobiological stress sensitivity a) interfere with crucial cognitive integrative functions, and b) are positively associated with increased threat avoidance behavior.

In the next section we will discuss the results of the studies presented in Chapters 2-6 in relation to these hypotheses, followed by a section in which these results will be theoretically integrated.

Cognitive threat sensitivity

The increased attentional bias for angry faces in patients with PNES, reported in Chapter 2, is in line with the first hypothesis that 'patients with PNES would show increased cognitive threat sensitivity'. For social threat cues (angry facial expressions) patients with PNES showed increased interference during a simple color-naming task when the colored targets were preceded by an angry face. In interpreting these results, it is important to emphasize that the face stimuli were presented subliminally, i.e. presented for only 14 ms before they were backwardly masked. The results of a so-called awareness check administered after the experiment, confirmed that both patients and HCs were not able to indicate the facial expression at a rate above chance level, probably reflecting that participants were merely guessing,

making it unlikely that subjects exerted strategic effort to control possible outcome effects (e.g. MacLeod & Hagan, 1992; Van den Hout et al. 1995; Williams et al., 1996; Putman et al., 2004). This is the first study to investigate the attentional processing of threat cues in patients with PNES. The results indicate that, already in the early stages of pre-attentive processing, there is an automatic processing bias for social threat cues in patients with PNES. Interestingly, this bias was even more pronounced in patients reporting sexual trauma. We interpreted patients' automatic attentional bias towards social threat cues as reflecting a state of hypervigilance in patients with PNES. Such vigilance for trauma-relevant stimuli is considered as a tendency to constantly scan the environment for any signs of potential threat (Buckley et al., 2000) or it could reflect an impaired suppression of trauma information once it is activated (McNally, 1998).

The attentional processing of the threat cues normalized after stress-induction. A possible explanation for this normalization in a stress-context will be provided later under the heading 'Towards an experimental model'.

Neurobiological stress sensitivity

The second hypothesis describing that 'Patients with PNES display increased neurobiological stress sensitivity' has been extensively tested in the current thesis. We will start by discussing the results of the HPA-axis, followed by results of other physiological stress parameters.

Basal cortisol. Chapter 3 offers support for increased basal cortisol levels in patients with PNES. In this study salivary cortisol was assessed while participants were in their own environment, outside the laboratory, and participants were explicitly instructed to participate on relative stress-free days. Results indicated that patients with PNES displayed increased levels of basal diurnal cortisol compared to HCs. These effects remained when relevant factors such as acute seizures, depressive symptoms, use of psychotropic medication and smoking behavior were statistically controlled for. The basal hypercortisolism in patients with PNES was furthermore particularly pronounced in those patients who reported a history of sexual trauma. These findings extend previous conflicting results with respect to basal cortisol levels in patients with PNES reported by Tunca and colleagues (1996; 2000) pointing at increased and not increased basal cortisol levels in patients with PNES, respectively. Besides methodological differences, such as the

fact that they sampled cortisol in serum and plasma instead of saliva (as we did) and that they applied a lower sampling rate of just a few time-points, they did not systematically control for possibly confounding factors, nor did they investigate the relation with psychological trauma reports. Therefore we tend to reconcile with Tunca et al. (2000), and conclude that patients with PNES showed increased basal cortisol levels, independent of the presence of current attacks.

Results from the short baselines measured in our laboratory studies were less conclusive. The salivary cortisol results described in Chapter 5 pointed again at a statistical trend towards increased basal cortisol levels in patients with PNES compared to HCs, whereas results from our study in Chapter 2 pointed at no difference in baseline cortisol levels between PNES and HCs. These inconsistencies within basal cortisol testing in the laboratory may be associated with increased stress related to a new situation and the artificiality of a laboratory setting, possibly resulting in less reliable baseline cortisol registrations.

In sum, based on the findings on basal cortisol described in Chapter 3 we conclude that patients with PNES display basal hypercortisolism which may be indicative of increased neurobiological stress sensitivity.

Cortisol stress-response. Salivary cortisol responses to a social and a physiological stress-induction protocol have been described in Chapters 2 and 5, respectively (note that Chapter 6 reports on a subsample from the sample described in Chapter 5). Results consistently showed that the cortisol stress-responses of the patients with PNES were comparable to those of the HCs. Interestingly however, the normal cortisol stress-response of the PNES patients reported in Chapter 5, occurred on top of slightly increased basal cortisol levels, resulting in a statistical trend for increased cortisol levels in patients not only at baseline, but throughout the experiment, including after stress-induction. Thus although the stress-responsiveness itself was not altered in PNES patients, the patients' absolute post-stress cortisol levels were somewhat inflated.

Dexamethasone-Suppression-Test (DST). HPA-axis self-regulatory functions were tested using a 1 mg DST. Initially, post-DST cortisol levels seemed to be increased in patients with PNES, but this group difference disappeared when controlling for group differences in smoking behavior. A possible explanation for this may be found in the increased enzyme-inducing effect in patients with PNES associated with their

increased smoking behavior, resulting in a faster drop of circulating dexamethasone in patients compared to HCs.

Cortisol Awakening Response (CAR). Patients' cortisol levels immediately after awakening were comparable to the CAR displayed by HCs.

Heart Rate Variability (HRV). In Chapter 2, further indications of increased (neuro)biological stress sensitivity have been established by the finding of decreased basal HRV in patients with PNES at baseline and in the recovery phase, often taken as an indication of hyperarousal (see e.g. Thayer & Brosschot, 2005, for a review).

Sympathetic nervous system (SNS). Additionally, basal concomitant SNS activity such as blood pressure, heart rate and noradrenergic activity (saliva alpha-amylase) as described in Chapters 2-3 and 5 have not been found to be increased in patients with PNES compared to HCs. These findings suggest that the present indications of increased neurobiological stress sensitivity are not merely due to patients' increased exposure to acute stress, but rather depicts a basal deregulation of the associated (neuro)biological stress systems.

To summarize: based on the results of increased basal cortisol levels and decreased HRV levels in patients with PNES, we concluded that PNES patients demonstrate increased basal activity in relevant stress systems, which cannot be attributed to increased levels of sympathetic activity related to acute stress (or seizures). These findings may point a basal deregulation of the associated (neuro)biological stress systems in patients with PNES.

Additionally, although this was not explicitly stated in our hypotheses, in the next paragraph we briefly describe a study investigating whether the increased cognitive and neurobiological stress sensitivity in patients with PNES were positively interrelated.

Integrating cognitive and neurobiological stress sensitivity

As is described in Chapter 4, basal pre-task cortisol was positively associated with the attentional threat vigilance described in Chapter 2 in patients with PNES, not in HCs and patients with epilepsy. Although basal pre-task cortisol in patients with PNES was not elevated compared

to both control groups, these results indicate that within the PNES patient group, patients with higher basal pre-task cortisol levels, also demonstrated increased attentional interference by the masked social threat cues.

Cognitive integrative functions

The first part of the third hypothesis states that patients' threat vigilance and increased basal cortisol levels interfere with important cognitive integrative functions. Contrary to our expectations, results in Chapter 5 demonstrated that basal WM performance in patients with PNES was not only impaired by social threat distracters (pictures of angry faces), but also by other social distracter pictures (pictures of happy and neutral faces as well). Because we did not present additional nonsocial distracters, we cannot determine whether WM performance in patients with PNES was impaired by the social disposition of the distracters or by the presence of distracters per se.

Interestingly, after stress-induction the no-distracter WM functions of HCs improved, but such improvement was not present in patients with PNES, resulting in a generalization of group differences in WM impairment. The improvement of general WM functions in HCs following a mild stress-induction is in line with a previous study of Lupien et al. (1999) demonstrating that mild doses of exogenous cortisol administration had beneficial effects on WM performance, alike the improvement of WM performance in HCs following stress-induction. Besides these beneficial effects of cortisol on WM performance, Lupien furthermore demonstrated an inverted U shaped curve for the effect of cortisol on WM performance, where relatively low and high levels of exogenous cortisol had negative effects on WM performance (Lupien et al., 1999). This model may offer an explanation for the present findings. PNES patients, who demonstrated a normal cortisol stress-response on top of their slightly elevated basal cortisol levels, did not show a relative improvement of WM performance following stress as the HCs did on the no-distracter condition. And although we did not find a relation with increased basal cortisol, within the patient group a high cortisol stress-response was associated with increased errors in the no-distracter condition. This relative worsening of general (no-distracter) WM performance may reflect an analogue for a more total collapse of the cognitive system following stress, associated with stress-induced cortisol.

Threat avoidance behavior

Finally, threat avoidance behavior is considered as an important etiological factor for PNES. Evidence for this theory is largely build on self-report investigations and no studies have systematically tested actual avoidance tendencies in patients with PNES yet. In line with the second part of the third hypothesis, we tested automatic threat avoidance tendencies in patients with PNES in relation to stress and cortisol levels. The results described in Chapter 6 demonstrated that patients showed a relative slowing when approaching angry faces compared to avoiding these social threat cues. These findings provide an objective confirmation of previous suggestions from self-report studies indicating that patients with PNES show relatively increased avoidance behavior in response to threat. In terms of the relationship between cortisol and threat avoidance tendencies, across groups, a positive association between pre-task baseline cortisol and the congruency-effect for angry faces was present. These results are in line with previous findings positively linking increased social threat avoidance behavioral tendencies to basal cortisol (Roelofs et al., 2005a; 2009a; Van Peer et al., 2007; 2009) and the third hypothesis positively linking basal cortisol and threat avoidance behavior. However, when testing this relation in both groups separately, the correlations were no longer significant. This lack of statistical significance in the patient group may be related to inadequate statistical power to detect small to medium correlation coefficients in a group of only 12 participants. The relative slowing for the threat approach trials in patients with PNES, was no longer present following stress-induction. A possible explanation for this normalization in a stress-context will be provided later under the heading 'Towards an experimental model'.

Theoretical integration

The biopsychosocial model of Reuber revisited

At this point we will re-examine our main findings in order to extend and specify some important aspects of the descriptive biopsychosocial model for PNES described by Reuber (2009; for an adapted version of this model, see Figure 7.1). First, Reuber stated that (early) psychological trauma and negative life events are important predisposing and precipitating factors for the development of PNES. Our studies consequently confirmed that psychological trauma may play a

role in PNES, but the current results specified that this was particularly so for sexual trauma, and we found no evidence for a relation with emotional or physical trauma. In addition to Reubers' model, we furthermore investigated how such an external predisposing factor may be associated with an internal vulnerability for PNES. Our results indicated a positive association between sexual trauma and increased cognitive and neurobiological stress sensitivity in patients with PNES. And although the present findings did not demonstrate how these external and associated internal factors are related to the development of the symptom PNES, they seemed to suggests that the presumed effect of psychological trauma on PNES may at least be partially mediated by an increased cognitive and neurobiological stress sensitivity in patients with PNES.

Secondly, based on self-report studies, Reuber (2009) further assumed that patients' avoidance behaviors to deal with threatening and conflicting situations form an important perpetuating factor in PNES. With the present research we were able to objectively confirm the previously published self-reports on increased avoidance behavior in response to threat in patients with PNES (Frances, 1999; Goldstein, 2000; 2006; see also Reuber, 2009). We did not directly test the assumption that threat avoidance behavior perpetuates PNES (Reuber, 2009). It seems likely, however, that recurrent use of avoidant coping leads to a failure to engage in adequate problem solving coping. As a result of this negative learning experience, patients are even less able to deal with threat and are more likely to perceive objectively harmless situations as severely stressful (Lazarus and Folkman, 1984, see Frances et al., 1999), thereby creating a negative spiral in which avoidant coping behavior in turn results in an increased threat perception.

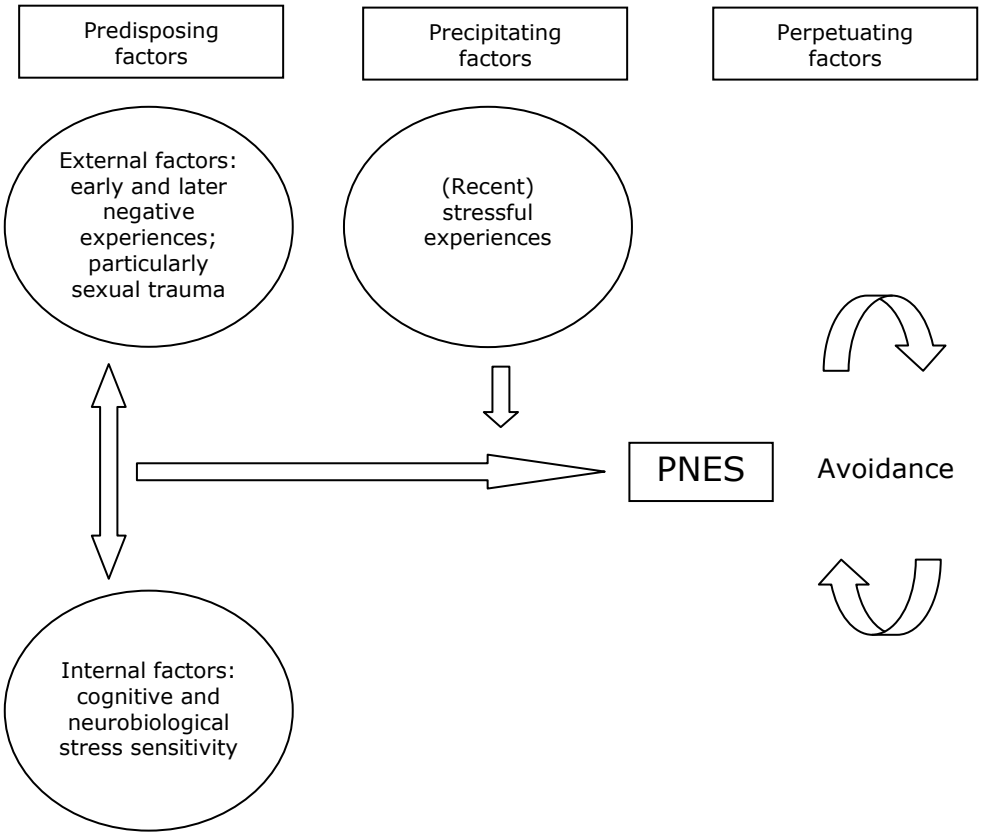

Figure 7.1. An adapted version of Reubers' multifactorial biopsychosocial model (Reuber, 2009).

Towards an experimental model of PNES

As is stated in Chapter 1, (neo-) dissociation theorists regarded PNES as cognitive-related complaints due to psychological stress factors (Janet, 1907; Hilgard, 1977; Kihlstrom, 1992; Brown, 2004). Using an integrative cognitive and neurobiological approach, as was suggested by Roelofs and Spinhoven (2007), we propose a partial experimental model of PNES describing how stress-induction may result in paroxysmal cognitive impairments in patients with PNES, which mimic presumed central cognitive processes during PNES. In this context the term experimental model denotes the reliable production of essential features of the disorder under study in standardized laboratory conditions. By manipulating independent conditions (such as inducing stress) the effects of this manipulation on dependent variables (such as cognitive

functions central to the disorder) can be studied in vulnerable participants in comparison to HCs.

The current findings of an attentional bias towards threat cues, decreased HRV and hypercortisolism in patients with PNES at baseline form accumulating evidence that basal cognitive and (neuro)biological systems of this patient group are under high amounts of strain. Some of these internal vulnerability factors were positively interrelated in patients with PNES, linking high neurobiological activity to increased cognitive hypervigilance in patients with PNES. Indications of increased basal cognitive strain are further evidenced by the observed generalization of WM impairments by, not only threatening social distracters, but also by nonthreatening (positive and neutral) social distracters. This effect was specific for PNES patients and already manifested in baseline test conditions (prior to any formal stress-induction). Furthermore, over and above their increased basal cortisol levels, patients demonstrated a normal cortisol stress-response, resulting in somewhat increased cortisol levels following stress-induction. The cortisol stress-response was in turn positively related to a further collapse of WM performance, a generalization of the relative WM deficits to the no-distracter condition. Such negative effects of high levels of cortisol on WM performance are in line with previously reported negative effects of both high levels of exogenous (Lupien et al., 1999; Oei et al., 2009) and endogenous (Elzinga & Roelofs, 2005) cortisol on WM.

This stress-induced generalization of WM impairment, associated with the cortisol stress-response in patients with PNES, may provide a fruitful partial experimental model for the phenomenon of a paroxysmal stress-induced disintegration of cognitive functions associated with the symptomatology of PNES. Based on the current findings, we suggest that it is the high basal activity of cognitive and (neuro)biological systems that makes a patient with PNES vulnerable for a paroxysmal disintegration of important cognitive functions under the added strain of a (normal) stress-response associated with a stress-context.

In retrospect, this proposed experimental model may also explain two unanticipated findings in Chapters 2 and 5. In those chapters we found that the cognitive threat vigilance and increased avoidance behavior in patients with PNES observed at baseline were no longer present in a

stress-context. However, if stress-induction and concomitantly stress-induced cortisol results in a collapse of general important cognitive integrative functions, it may very well be that patients were simply no longer able to register the (threat) valence of the social stimuli appropriately in the stress contexts described in Chapters 2 and 5.

Before discussing the specificity of this proposed model, it is important to note that the stress-inducing protocols used in our study did not actually provoke the symptom PNES, but merely resulted in a paroxysmal cognitive impairment. Our research, therewith, only covers possibly relevant cognitive aspects associated with PNES, leaving the matter of the semiology untouched. As a result we only provide a partial experimental model for PNES covering some general cognitive integrative functions during PNES. Although the motoric features associated with PNES are beyond the scope of the present thesis, theorizing and speculating about the motoric components associated with PNES is highly interesting. Several preliminary premises have been formulated in the field of biological, cognitive and social learning theories in an attempt to explain the motoric features associated with PNES. Kretschmer for instance (1926, as described in Ludwig, 1972) introduced phenomena as 'sham-death' and 'violent motor reaction' as possible biological mechanisms underlying symptoms such as PNES. More recently, Brown (2004) formulated his cognitive hierarchical theory, in which he submitted that activation of unattended lower level cognitive systems may be associated with physiological symptoms such as PNES. Moreover, Bautista et al. (2008) recently focused on the automatic processes related to social learning in patients with PNES, with their research on symptom modeling in patients with PNES (without comorbid epilepsy). These viewpoints are a few examples of a wide range of intriguing but preliminary theories on the motoric components of PNES. A logical next step would be to further develop these assumptions on the motoric features of PNES into testable hypotheses to ultimately integrate a PNES model for the motoric features with the presently described cognitive and neurobiological aspects of PNES to gain more insight into the synergic mechanisms underlying PNES.

Specificity of the experimental model

Deregulation of the HPA-axis and (its associated effects on) cognitive integrative functions such as WM have been extensively studied in patient populations over the last few years. Along the lines of (neo-) dissociation theorists who regarded PNES as a dissociation of important cognitive functions in response to psychological stress factors (Janet, 1907; Hilgard, 1977; Kihlstrom, 1992; Brown, 2004) we will now evaluate the specificity of our experimental model in relation to previous findings in other relevant trauma-related disorders with dissociative features. Because WM deficits have been reported to play a central part in the mechanisms underlying both Post Traumatic Stress Disorder (PTSD) and dissociation disorder, we will focus on these patient groups when discussing the specificity of the presently described neurobiological and cognitive impairments in patients with PNES.

PTSD. Although PTSD is by definition associated with a history of psychological trauma, the associated alterations of HPA-axis activity seem to oppose our findings in patients with PNES. In a systematic review and meta-analysis on basal cortisol levels in adult PTSD patients, Meewisse et al. (2007) reported *lower* basal afternoon cortisol in female PTSD patients, particular in those patients reporting early sexual or physical trauma. Also, cortisol hyper-suppression following DST, resulting in reduced cortisol levels following DST, has been reported in PTSD. Studies using non-pharmacological stress paradigms, such as cognitive stress or trauma reminders to stimulate the HPA-axis, showed an exaggerated cortisol response in patients with PTSD (for a review see de Kloet, 2006).

PTSD is generally characterized by WM deficits (e.g. Galletly et al., 2008; Veltmeyer et al., 2009). Moreover, one study investigated the effect of neutral and trauma-relevant distracters on WM performance in patients with PTSD and illustrated increased WM impairment by both neutral and trauma-relevant distracter types (Morey et al., 2009). To our knowledge, so far no studies have investigated the effect of stress-induction paradigms on WM performance in patients with PTSD. Two studies investigating the effect of (comparable doses of) cortisol administration on WM in PTSD led to contradictory results, one study reported cortisol-induced WM impairment (Grossman et al., 2006), while the other study reported a WM enhancement following cortisol administration in patients with PTSD (Yehuda et al., 2007). In sum, in contrast to PNES, the cortisol patterns generally displayed by patients

with PTSD are characterized by basal *hypo*cortisolism and an *increased* cortisol stress-response. PTSD patients' general WM impairment and increased WM interference of both trauma-relevant and neutral distracters are however in accordance with our study results in patient with PNES.

Dissociative disorder. Our findings of basal hypercortisolism in PNES patients resemble previous findings in patients with a primary dissociative disorder. Increased basal 24-hour urine cortisol was found in 46 patients with Dissociative Identity Disorder (DID) compared to HCs (Simeon et al., 2007). Post-DST cortisol was also increased in these DID patients as well as in 9 patients with Depersonalization Disorder (Simeon et al., 2001). Cortisol stress-response in the DID patients was unimpaired (Simeon et al., 2007). Based on this scarce evidence, one might hypothesize that the HPA-axis hyperactivity in PNES patients shares more overlap with dissociative disorder than with PTSD. However, in contrast to its similarity with PNES on neurobiological ground, available studies indicate that WM performance in pathological dissociation may be rather different from patients with PNES. A recent study by Elzinga et al. (2007) indicated that, compared to HCs (no-distracter) WM performance in 13 patients with DID was slightly worsened at relative low task loads but somewhat enhanced with increasing task load (although these results did not remain significant when corrected for multiple comparisons). The authors interpreted these results as an enhancement of WM in patients with DID when the task is sufficiently demanding (Elzinga et al., 2007). Effective cognitive performance, including intact threat inhibition, in demanding circumstances is furthermore stated by Dorahy (2006) describing that, in a threat context, high dissociators have the capacity to function effectively with multiple streams of processing in operation, also outside consciousness (Hilgard, 1986; 1994; DePrince & Freyd, 2001, see Dorahy, 2006).

To our knowledge, no studies have yet investigated the effect of (stress-induced) cortisol on WM performance in patients with dissociative disorders. The WM enhancement in sufficiently demanding circumstances associated with dissociation is in contrast with the present results of increased WM impairment by general social distracters at baseline and following stress-induction, irrespective of cognitive load in patients with PNES. Elzinga et al. (2007) stated that "Whereas PTSD [and PNES] is assumed to involve a breakdown of WM, probably due to

insufficient inhibition of trauma-related thoughts and feelings, dissociative patients may be characterized by strong executive control capacities, thereby inhibiting the processing of trauma-related memories. In dissociative patients, this may take place at the expense of other functions that require attention, however, such as a sense of personal identity and reality, inducing feelings of depersonalization and derealization" (Elzinga et al., 2007, p 243).

To conclude: placing the present findings in a spectrum of relevant trauma-related disorders with dissociative features, indicates that patients with PNES share overlap with dissociative disorders with respect to basal neurobiological hyperactivity, but share the intrusive WM breakdown which has been previously described in patients with PTSD. Based on the outcome that the overall pattern of interrelated cognitive and neurobiological impairments in patients with PNES does not immediately fit with other relevant disorders in this spectrum, leads us to hypothesize that the proposed pathophysiology may be specific for PNES. Testing the similarities and commonalities in cognitive and neurobiological processes among trauma-related disorders with dissociative features constitutes a fruitful area for further research that may provide relevant new insights in the pathophysiology of these different symptom manifestations.

Strengths and limitations and suggestions for future research

Before discussing possible clinical implications of the studies presented in this thesis, some strengths and limitations and suggestions for future research will be provided. The primary strength of the present research is the application of an experimental approach. Although this approach has become quite common in the study of other trauma-related disorders with dissociative features, application of this approach in the field of PNES is still in its infancy. This experimental laboratory approach, including the extensive registering of several physiological stress parameters, enabled us, in addition to self-report studies, to objectively test the effects of threat stimuli and a stress context on relevant cognitive and behavioral functions and relate them to trauma and neurobiological activity in patients with PNES (Roelofs and Spinhoven, 2007). This methodology proved to be a valuable tool to test, specify and extend the existing literature on patients with PNES. Another important strength of the present research is that all patients

were diagnosed using the gold standard: an ictal video-EEG registration of a typical seizure in order to confirm the absence of epileptiform activity, making PNES diagnosis maximally reliable (for reviews see e.g. Reuber and Elger 2003; LaFrance et al., 2008). In addition, it is positive that the HCs in our studies were similar to patients with PNES on many relevant demographic variables, such as gender, age, educational level and use of contraceptives and menstrual cycle (for cortisol assessment), minimizing the biasing effect of random factors on the described results. Additionally, patient characteristics such as use of psychotropic medication, has been dealt with by including only patients not using psychotropic medication in one study (Chapter 2). However, because of reduced generalization to patients taking medication we subsequently choose to statistically control for psychotropic medication use in patients with PNES. Also, a total of 3 patients were excluded from testing (2) or all analyses (1) due to one or more seizures on test days, interfering with the assessments. In Chapter 4, 3 more patients who reported the occurrence of a seizure prior to saliva cortisol sampling were excluded from a reanalysis, ensuring that the basal hypercortisolism in patients with PNES was not merely due to (increased stress or physiological movements associated with) the acute occurrence of PNES.

Based on a recent discussion suggesting that poor neurological functioning in patients with PNES might be associated with poor effort during task performance (Cragar et al., 2006; Drane et al., 2006; Locke et al., 2006; Dodrill et al., 2008), we investigated indications of poor effort by the administration of a malingering task which was presented to participants as a memory task, when task performance was under direct influence of the amount of effort deployed by the participant, as was the case for the N-back task (Chapter 5) and the AA-task (Chapter 6). Based on this malingering task, we excluded 1 patient based on suspicion of task-underachievement (in Chapter 6 this person was already excluded from analyses due to technical failure; this is not explicitly mentioned in Chapter 6). Moreover, subsequent response pattern analysis of the N-back task, which is considered as a difficult task, lead us to exclude 5 more patients and 2 HCs because their response patterns indicated noncompliance, illustrating the importance of studying effort and compliance in (complex) cognitive experiments.

An important limitation of the present studies is the lack of a clinical control group, making it difficult to state the specificity of the described effects for PNES patients and to exclude the possibility that

these effects were mediated by comorbid psychopathology. Although it is a less elegant solution than including a clinical control group, we statistically controlled for group differences in anxiety and depressive symptoms in the studies described in Chapters 3 and 5-6 by reanalyzing group effects with anxiety and depressive symptoms included as covariates into the analyses. And although we did not report these results in Chapter 2, similar reanalyzes of the basal emotional Stroop data with anxiety and depression scores (SCL-90 R) as covariates, demonstrated similar patterns of the basal emotional Stroop data as described in Chapter 2 [Valence X Group: $F(1,35)=4.15$, $p=.049$)]. Based on the results of these reanalyzes, we are reasonably confident that the findings described in this thesis are not merely due to group differences in anxiety and depressive symptoms.

Another limitation is that we, except in Chapter 4, did not report data of an epilepsy control group, in order to control for having a chronic and severely disabling disorder that strongly resembles PNES. As was stated in Chapter 1, we included patients with epilepsy in our research, but along the way we concluded that patients with epilepsy form a heterogeneous group due to a variety of patient characteristics (e.g. type of seizure(s), epileptogenic focus, use of (often multiple) AEDs, lower educational status) which were of relevance to the cognitive and neurobiological functions we tested, making them unfit as a control group. We also did not include patient with PNES and concurrent epilepsy in our studies. Consequently, we cannot generalize our finding to PNES patient with comorbid epilepsy.

Additionally, interpersonal trauma rates were based on a self-report questionnaire and were not further verified using independent sources. Relying solely on independent sources, however, would probably have resulted in an inclusion bias of patients who stood up and explicated what happened to them, which may result in a different sample excluding the more 'silent sufferers'. It can furthermore be conceived that the use of this criterion would result in a selection bias of shorter and less severe types of trauma (by unknown persons) vs. long lasting abuse by significant others (e.g. family members).

Finally, in our research, we did not control for the effect of psychological treatment. In some participants PNES was diagnosed recently and patients had not (yet) received any psychological treatment. But patients with PNES who had successfully finished their psychological treatment and reported to be seizure-free for a few

months were also included in this research project (as long as they had 2 PNES in the year prior to participating). Obviously, future research should control for psychological treatment, preferably by a prospective study with pre-treatment, post-treatment and follow-up assessments. An alternative would be a cross-sectional design including groups of patients at the beginning of psychological treatment and compare their results to patients in the end-stage of psychological treatment and a follow-up group. Seizure frequency may serve as a parameter for treatment success. Other characteristics such as age of onset and duration of PNES (disorder, not seizure) are relevant factors within patients to (statistically) control for (Black et al., 2010). Alternatively, there is a clear need to explore the association of patients' cognitive and neurobiological impairments with their symptomatology, by assessing patients before and after (successful) treatment.

Besides the suggestions for future research that arise from the section 'limitations', more suggestions for future research are provided below. Reuber (2009) specified early psychological trauma as an important predisposing factor and (additional) negative life events later in life as a precipitation factor for the development of PNES. To further specify the effect of trauma, it would be helpful to investigate cognitive and neurobiological stress sensitivity in patients with PNES including a large group of patients with PNES reporting a history of (early) psychological trauma versus a subgroup of PNES patients without such a trauma report. It would furthermore be interesting for future studies to investigate whether early trauma makes patients' central stress system more vulnerable to the effects of later stressors, that in turn serves as a precipitating factor for PNES onset (Reuber, 2009, see also Roelofs & Spinhoven, 2007). Also, our results pointing to the specific effect of sexual trauma, and not emotional trauma or physical abuse in general, on increased attentional threat processing and basal hypercortisolism in patients with PNES, are in line with suggestions of Selkirk et al. (2008) that PNES patients reporting sexual trauma are more severely impaired than patients with PNES without sexual trauma reports, but need further replication.

Secondly, extending the high rates of psychological trauma commonly reported in patients with PNES, we believe that it would be very interesting for future research to test patients' stress-response using autobiographical trauma scripts as a stress-induction tool. The use

of personalized trauma scripts may constitute a more relevant or specific stressor compared to the Trier Social Stress Test (TSST) and the Cold Pressor Test (CPT), perhaps yielding different results (e.g. Elzinga et al., 2003). Alternatively, to test the specific effect of cortisol-induction on cognitive integrative functions, without concomitant SNS activation, the administration of exogenous cortisol may be considered for future studies in patients with PNES (Lupien et al., 1999).

Thirdly, we are the first to experimentally study the cognitive processing of emotional stimuli in patients with PNES. On account of the presumed relevance of commonly reported interpersonal traumatic experiences in patients with PNES, we tested the effects of social stimuli in the form of photos of facial expressions. Because we did not include nonsocial emotional stimuli, we cannot determine whether WM performance in patients with PNES was impaired by the social disposition of the distracters or by the presence of distracters in itself (Chapter 5). Future studies should therefore also study the cognitive interference effect of nonsocial distracters.

Finally, with regard to neural correlates, we already mentioned some brain structures that may be involved in mediating effects of stress on cognitive impairments in Chapter 5. These neural mechanisms have, however, never been tested in patients with PNES and important questions for future research are whether patients' increased social distracter WM interference and threat avoidance behavior may be associated with increased limbic processing or with decreased prefrontal inhibition of the social cues, or both. It would therefore be interesting for future studies to test patients' cognitive impairments and avoidance behavior and their neural correlates using brain imaging techniques.

Clinical implications

PNES occurs in a heterogeneous group of patients (e.g. Reuber, 2009) and there is no empirical evidence so far to suggest that a unified pathological mechanism underlies PNES. As a result, psychological treatment of PNES is often highly individualized. An effective treatment, leading to long-term seizure freedom in a high percentage of PNES patients, does not yet exist (Kuyk et al., 2008). So far no randomized controlled trials (RCT) have been performed to test the efficacy of different forms of PNES treatments, and as a result no generally accepted treatment protocol currently exists.

Based on the empirical findings in PNES described in this thesis, we formulated a partial experimental model for PNES, describing how internal cognitive and (neuro)biological factors, which are related to external predisposing factors such as sexual trauma, make patients vulnerable for later stress-induced paroxysmal disintegration of cognitive functions, associated with PNES. This experimental model for PNES, although partial and hypothetical, may be useful in the development of new and more refined treatment approaches for PNES. Below we will provide some suggestions for clinical treatment of PNES.

As described earlier, we suggest that the relatively high basal levels of activity of both cognitive and (neuro)biological systems in patients with PNES, leave patients vulnerable for a paroxysmal disintegration of important cognitive functions under the added strain of a normal stress-response associated with a (perceived) stress-context. We therefore believe that it is essential to focus on these basal cognitive and neurobiological vulnerabilities, in order to prevent a general collapse of stress-induced cognitive functions, which are assumed to be characteristic for PNES.

Several approaches may be helpful here. First, in order to 'normalize' the social threat vigilance in patients with PNES, attentional retraining may be applicable in order to train patients to modify their attentional pattern to reduce vigilance for social threat (e.g. Dandeneau et al., 2007). Secondly, patients' hypercortisolism may be dampened by the administration of some antidepressants, which have been shown to be effective in lowering basal saliva cortisol levels in depressed patients (e.g. Scharnholz et al., 2010; for a review see e.g. Mason and Pariante, 2006). Additionally, because threat vigilance and basal cortisol levels were positively interrelated, it would be interesting to investigate whether modification of the attentional bias towards threat is associated with a decrement of basal cortisol levels in patients with PNES. Or alternatively, whether cortisol decrements would be associated with a normalization of threat vigilance, or whether such changes are unrelated in patients with PNES. It is important to emphasize that these interventions aim to normalize impaired cognitive and neurobiological functioning in patients with PNES, which we theorized to be internal factors leaving patients with PNES vulnerable for a stress-induced impairment of important cognitive functions. Testing their effects on the symptom PNES would be a crucial next step. Additionally, the objective

confirmation of previous self-report studies describing increased avoidant (coping) behavior in response to threat or conflicting situations, strengthens the existing literature on the importance of adequate problem solving coping skills in patients with PNES (Frances et al., 1999; Goldstein et al., 2000; 2006). Because avoidance behavior is considered to be an important precipitating and perpetuating factor for PNES (Reuber, 2009), it might be worth investigating whether changes in patients' self-reported coping strategies are indeed confirmed by changes in automatic threat avoidance tendencies after successful treatment. In addition, it would be clinically highly relevant to investigate whether (changes in) threat avoidance behavior could serve as a predictor for PNES prognosis.

Because psychological trauma is an important etiological factor in PNES, (imaginary) exposure to negative life events often forms an important part of the psychological treatment of PNES. Our present finding of a collapse of patients' important cognitive functions following stress-induction, argues for a phase-oriented treatment model, which has for instance been proposed by Lanius et al. (2010) for the treatment of a dissociative subtype of PTSD, associated with chronic abuse. This phase-oriented treatment model starts with the implementation of a stabilization phase, prior to starting with the trauma (imaginary) exposure therapy. We believe that such a treatment approach would be useful in patients with PNES, to avert a cognitive collapse associated with the stress-inducing effect of a premature trauma exposure therapy, preventing patients to benefit from this therapy.

In sum: the present thesis presents a novel approach integrating both cognitive and neurobiological research in order to test, specify and extend cognitive and stress-theories of PNES. Based on a series of (experimental) studies we propose a partial experimental model for the stress-induced cognitive impairments associated with PNES. This working model proposes that the high basal levels of activity in cognitive and (neuro)biological stress systems makes PNES patients vulnerable for a paroxysmal disintegration of important cognitive functions under the added strain of a normal stress-response associated with stress-induction. Major findings that contributed to this model were 1) the finding of increased cognitive threat sensitivity and the result of increased basal cortisol levels associated with (sexual) trauma (Chapters 2-3) and 2) stress and cortisol induced generalization of cognitive

impairments (Chapter 5). Relating these findings to previous findings in other relevant trauma-related disorders with dissociative symptoms, prudently suggests that the pathophysiological profile of PNES contains specific and unique elements. At present there is no generally accepted treatment protocol for PNES and the current findings may offer important starting points for the development of more tailored treatment strategies. The present results suggest that it may be fruitful for psychological and pharmacological treatment for PNES to include strategies to normalize the basal cognitive and neurobiological stress hypersensitivity in addition to the achievement of adequate stress coping strategies.

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Samenvatting

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Samenvatting

Psychogene Pseudo Epileptische Aanvallen (PPEAs; in het Engels Psychogenic Non Epileptic Seizures, afgekort PNES) is een aandoening die gekenmerkt wordt door op epilepsie lijkende aanvallen waarvoor geen neurologische of andere organische oorzaak gevonden wordt. De aandoening wordt beschouwd als een paroxysmale desintegratie van cognitieve functies die geassocieerd is met psychologische stressfactoren.

Naar schatting wordt voor 20% van de patiënten die zich aanmeldt bij gespecialiseerde epilepsieklinieken geen organische oorzaak gevonden voor de aanvallen en bij een groot deel van deze patiënten is er sprake van PPEAs. Ondanks de hoge frequentie en de ernstige beperkingen die deze stoornis met zich meebrengt, is er weinig bekend over de aard en de etiologie van deze stoornis. Hoewel PPEAs van oudsher worden geassocieerd met psychologische trauma's en stress, is de relatie tussen trauma en stress enerzijds en cognitieve functies anderzijds, nog niet systematisch onderzocht bij mensen met PPEAs. Het doel van dit promotieproject was om inzicht te verschaffen in de mogelijke effecten van psychologische en neurobiologische stress-factoren op cognitieve en gedragsmatige aspecten van emotionele informatieverwerking bij patiënten met PPEAs. De theoretische inkadering van dit proefschrift baseert zich enerzijds op bevindingen uit zelfrapportage onderzoek waaruit blijkt dat patiënten met PPEAs meer psychogene trauma's en stressfactoren rapporteren dan gezonde controles, patiënten met epilepsie en zelfs patiënten met affectieve stoornissen. Op de tweede plaats baseert dit proefschrift zich op bevindingen uit dierexperimenteel en humaan onderzoek waaruit blijkt dat vroege traumatisering gepaard kan gaan met langdurige verhoging van stressgevoeligheid in belangrijke biologische stresssytemen als het autonome zenuwstelsel en met name de hypothalamus hypofyse bijnier as (HPA-as). Tenslotte wordt verhoogde sensitiviteit in deze stresssystemen in verband gebracht met verstoringen van complexe cognitieve functies, zoals werkgeheugen en het kunnen inhiberen van irrelevante informatie. Beide aspecten lijken in de klinische praktijk verstoord tijdens het voorkomen van de PPEAs.

De centrale hypothese van dit onderzoeksproject was dat patiënten met PPEAs een verhoogde stressgevoeligheid laten zien, die op zijn

beurt kan leiden tot paroxmale desintegratie van belangrijke cognitieve functies. Meer concreet werden de volgende hypotheses getoetst:
1). Patiënten met PPEAs vertonen een cognitieve bias voor (sociaal) dreigende informatie.
2). Patiënten met PPEAs vertonen een verhoogde (neuro)biologische stressgevoeligheid.
3). Deze verhoogde cognitieve dreigingsgevoeligheid en neuro-biologische stressgevoeligheid leiden tot verstoringen in integratieve cognitieve en gedragsmatige functies. We verwachten a) een verstoring van belangrijke cognitieve integratieve functies zoals werkgeheugen, en b) toegenomen vermijding van (sociaal) dreigende informatie bij patiënten met PPEAs.

De belangrijkste onderzoeksuitkomsten van deze dissertatie zullen aan de hand van bovenstaande voorspellingen worden besproken.

Cognitieve dreigingsgevoeligheid

Hoofdstuk 2 beschrijft een studie waarin we onderzochten of patiënten met PPEAs gevoelig zijn voor sociale dreiging. We richtten ons hierbij met name op de pre-attentieve verwerking van sociaal dreigende stimuli. Patiënten met PPEAs en gezonde controles kregen subliminale gezichten aangeboden in een aangepaste gemaskeerde emotionele Stroop taak, die zowel in een baseline (rust) conditie werd afgenomen als in een stressconditie. In deze reactie-tijden (RT) taak werden foto's van boze, blije en neutrale gezichten slechts 14 ms aangeboden, waarna ze direct gemaskeerd werden door een neutrale eivormige afbeelding waarvan de kleur benoemd diende te worden. De aandachtsbias voor emotionele gezichten trials werd berekend door RTs van de neutrale gezichten trials af te trekken van de RTs van de emotionele gezichten trials. In de baseline conditie lieten de patiënten een aandachtsbias voor boze gezichten zien in vergelijking met de gezonde controles. Deze aandachtbias voor boze gezichten correleerde bovendien positief met rapportage van sexueel trauma in de patiëntengroep. Dit effect was specifiek voor boze gezichten, niet voor de blije gezichten, en was niet langer aanwezig na een sociale stress-inductie procedure.

Deze resultaten duiden op een aandachtsbias voor sociaal dreigende stimuli bij patiënten met PPEAs, reeds in de vroege fase van de aandachtsverwerking (voordat de gezichten expliciet waargenomen zijn), welke zich nog duidelijker aftekent bij patiënten die een seksueel

trauma rapporteren. Deze basale hypervigilantie voor dreiging bij patiënten met PPEAs biedt ondersteuning voor de hypothese dat patiënten met PPEAs een verhoogde cognitieve dreigingsgevoeligheid laten zien.

Verhoogde neurobiologische stressgevoeligheid

Om de tweede voorspelling -verhoogde neurobiologische stressgevoeligheid van patiënten met PPEAs- te toetsen hebben we de hypothalamus hypofyse bijnier (HPA)-as met het eindproduct cortisol in verschillende condities (baseline en na psychologische en fysiologische challenges) onderzocht. Hoofdstuk 3 beschrijft een studie waarin patiënten met PPEAs en gezonde controles op twee opeenvolgende en relatief stressvrije dagen speekselmonsters verzamelden in hun eigen leefomgeving (dus niet in het laboratorium), waaruit cortisol werd verzameld. De resultaten toonden aan dat de basale diurnale cortisolwaardes van patiënten met PPEAs verhoogd zijn in vergelijking met de gezonde controles, zelfs wanneer er rekening gehouden wordt met verschillende relevante variabelen, zoals acute aanvallen, medicatie, roken en depressieve symptomen. Ochtendcortisol en het zelfregulerend vermogen van de HPA-as, gemeten door de Dexamethason-Suppressie-Test, van de patiënten waren onaangedaan (Hoofdstuk 3), evenals de cortisolwaardes na een sociale stress-inductie (Hoofdstuk 2). In het stress-experiment dat beschreven staat in Hoofdstuk 5, waarin stress geïnduceerd werd middels een fysiologische stressor, de Cold Pressor Test, was er sprake van een statistische niet-significante trend voor verhoogde cortisolwaardes gedurende het hele experiment bij PPEA patiënten.

Op basis van deze resultaten kunnen we concluderen dat patiënten met PPEAs een verhoogde neurobiologische stressgevoeligheid vertoonden in de vorm van verhoogde basale cortisol. Bovendien waren de verhoogde basale cortisolwaardes van patiënten met PPEAs gerelateerd aan de verhoogde aandachtsbias voor sociaal dreigende stimuli, zoals beschreven in een extra analyse in Hoofdstuk 4. Tenslotte vonden we aanwijzingen voor een verlaagde hartslag variabiliteit (HRV) bij patiënten met PPEAs, een parameter welke geassocieerd wordt met arousal. In Hoofdstukken 2-3 en 5 worden geen aanwijzingen gevonden voor een verhoogde activatie van het sympatisch zenuwstelsel bij patiënten met PPEAs.

Cognitieve integratieve functies

Vervolgens hebben we getoetst of de verhoogde cognitieve dreigingsgevoeligheid en de verhoogde neurobiologische stress-gevoeligheid van patiënten met PPEAs invoed hebben op meer complexe, integratieve cognitieve functies. Om deze voorspelling te onderzoeken, hebben we een werkgeheugentaak afgenomen bij patiëntien met PPEAs en gezonde controles in een baseline (rust) en een stress conditie (Hoofdstuk 5). Het werkgeheugen is een cruciale integratieve cognitieve functie, welke noodzakelijk is voor bijna iedere vrijwillige actie. In een zogenaamde N-back taak, moesten deelnemers aangeven of een letter uit een letterreeks een bepaald aantal aanbiedingen eerder reeds getoond was. Eventuele groepsverschillen in het inhiberen van irrelevante (dreigende) informatie werden onderzocht door de letters al dan niet te plaatsen op een afleidende achtergrond (een boos, blij of neutraal gezicht). Deelnemers werden expliciet geïnstrueerd om deze gezichten op de achtergrond te negeren. Al in de baseline conditie maakten patiënten meer fouten dan de gezonde controles, niet alleen bij boze gezichten maar bij alle trials met een afleidende achtergrond, terwijl ze het op trials zonder afleidende achtergrond net zo goed deden als de gezonde controles. Na een fysiologische stress-inductie (hand in ijswater) verbeterden de werkgeheugenprestaties van de gezonde controles, maar niet van de patiënten, waardoor het groepsverschil generaliseerde naar de trials zonder afleidende achtergrond. Wat betreft de relatie tussen cortisol en werkgeheugenprestatie vonden we geen relatie met basale cortisol waardes, maar wel een positieve correlatie tussen de cortisol stress-respons van de patiënten en hun stress-geïnduceerde werkgeheugen-prestaties (aantal fouten) in de conditie zonder afleidende achtergrond.

Deze bevindingen lijken een eerste aanwijzing te zijn dat niet alleen dreigende sociale (gezichts)stimuli, maar alle gezichtsstimuli interfereren met cognitieve integratieve functies van patiënten met PPEAs. Ook geven de huidige resultaten aanwijzingen dat stress-inductie en de gerelateerde cortisol stress-respons samenhangen met een relatieve verstoring van integratieve cognitieve functies (zonder sociale afleiders) van patiënten met PPEAs.

Vermijdingsgedrag

Tenslotte wordt vermijdingsgedrag in de literatuur vaak genoemd als een belangrijke etiologische factor van PPEAs. Tot nu toe is vermijdingsgedrag van patiënten met PPEAs alleen onderzocht middels zelfrapportage onderzoek en nog niet met behulp van meer objectieve gedragsmaten. In hoofdstuk 6 hebben we een studie beschreven over de sociale vermijdingsgeneigdheid van patiënten met PPEAs en de relatie met stress en cortisol. Een computergestuurde manuele approach-avoidance (AA) taak werd afgenomen bij zowel patiënten met PPEAs als gezonde controles. De AA taak is een RT taak waarbij deelnemers foto's evalueren van blije en boze gezichten, door het maken van een toenaderende (arm flexie) of een vermijdende (arm extensie) armbeweging. Een affect-congruente respons bestaat uit het vermijden van boze en het toenaderen van blije gezichten en een affect-incongruente respons uit het vermijden van blije gezichten en toenaderen van boze gezichten. De taak werd, evenals de N-back taak, in een baseline en een stress conditie afgenomen. In rust vertoonden de patiënten voor de boze gezichten een beduidend sterkere vermijdings-geneigdheid dan gezonde controles. De patiënten lieten een relatieve versnelling zien in het vermijden (en niet het toenaderen) van boze gezichten. Voor blije gezichten werden dergelijke groepsverschillen in actiegeneigdheid niet gevonden. Deze effecten verdwenen na de fysiologische stress-inductie.

Wat betreft de relatie tussen cortisol en sociale approach-avoidance geneigdheid vonden we over beide groepen heen een positieve correlatie tussen baseline cortisol en het approach-avoidance effect voor boze gezichten.

Op basis van bovenstaande resultaten van verhoogde cognitieve dreigingsgevoeligheid en verhoogde neurobiologische stressgevoeligheid van patiënten met PPEAs en het effect hiervan op relevante cognitieve integratieve functies en vermijdingsgedrag, doen we in de discussie van het proefschrift een voorstel voor een partieel experimenteel model voor het fenomeen PPEAs. Dit model beschrijft hoe stress-inductie kan resulteren in een paroxysmale desintegratie van cognitieve integratieve functies. Het is belangrijk om te benadrukken dat het een *partieel* model betreft, aangezien het zich strikt beperkt tot (één van) de cognitieve componenten van een PPEA, en de motorische en fysiologische kenmerken van een aanval geheel buiten beschouwing laat.

Het partiële experimentele model voor PPEAs stelt, naar aanleiding van bovenstaande resultaten, dat niet de stress-inductie op zichzelf leidt tot een desintegratie van belangrijke cognitieve functies, maar dat een normale stress-respons bovenop de reeds verhoogde cognitieve dreigingsgevoeligheid en verhoogde basale activatie van het neurobiologische stresssysteem de condities schept waarin een desintegratie van relevante cognitieve functies kan plaatsvinden. De verhoogde basale activiteit van cognitieve en neurobiologische stress systemen maken een PPEA patiënt mogelijk kwetsbaar voor een paroxysmale desintegratie van belangrijke cognitieve functies onder de toegenomen druk van een normale stress-respons geassocieerd met een stress-inductie.

Vergelijkingen van het profiel van de resultaten bij patiënten met PPEAs met eerdere bevindingen bij relevante trauma-gerelateerde aandoeningen met dissociatieve symptomen, zoals Post-Traumatische Stress Stoornis of Dissociatieve Stoornis, lijken erop te wijzen, dat het pathofysiologische profiel van PPEAs specifieke en unieke elementen bevat. Dit biedt mogelijk nieuwe aanknopingspunten voor een specifieke behandelwijze van PPEAs. De huidige onderzoeksresultaten wijzen erop, dat het voor de psychologische en farmacologische behandeling van PPEAs mogelijk lonend zou zijn om strategieën te includeren die de verhoogde basale cognitieve dreigingsgevoeligheid en neurobiologische stressgevoeligheid normaliseren, bovenop het aanleren van adequate coping strategieën om beter met stress en dreiging om te gaan.

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CV

Patricia Bakvis (Pijnacker, 1976) graduated from high school at the Albeda College in Rotterdam in 1999, after which she started to study psychology at Leiden University. She obtained her Masters degree in August 2004. As part of her study, she did an extended clinical and research internship at Stichting Epilepsie Instellingen Nederland (SEIN) in Heemstede, studying the effects of trauma and stress on cognitive functioning in patients with Psychogenic Non Epileptic Seizures (PNES), under supervision of Dr. Karin Roelofs, Dr. Peter de Heus, and Dr. Jarl Kuyk. This study formed the basis for a Teding van Berkhout Fellowship grant awarded to Patricia Bakvis by de Christelijke Vereniging voor de Verpleging van Lijders aan Epilepsie, allowing her to conduct a full PhD research project. The current dissertation, under the supervision of Prof. Dr. Karin Roelofs, Prof. Dr. Philip Spinhoven and Prof. Dr. Frans Zitman forms the result of this project, which was embedded in the Dutch-Flemish research school Experimental Psychopathology (EPP). In addition to her research activities, Patricia Bakvis has been working as a psychologist, treating patients with PNES and epilepsy, in SEIN Heemstede.

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