The effect of hydrocortisone on fear information processing and fear extinction

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Thesis submitted for the degree of Master in Science (by Research) in Psychiatry

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Michaelmas Term 2018

Introduction: Previous work has revealed that the success of exposure-based therapies in individuals with anxiety disorders can be i) enhanced with pre-treatment glucocorticoid administration and ii) predicted by raised endogenous cortisol levels during treatment. In recent years, computer-based exposure initiatives that minimise costly and time-intensive therapist input whilst providing greater access to treatment have been successfully developed. Given the benefits of these novel approaches, it is plausible that combining treatment ingredients could lead to additional gains. This study aimed to investigate for the first time the potential of hydrocortisone to enhance the efficacy of one-session, computer-based cognitive behavioural therapy (CBT) in participants with high spider fear. It also aimed to determine whether glucocorticoid-enhanced outcomes were predicted by early alterations to the fear bias, which has previously been demonstrated to be a key mediator and predictor of symptomatic improvement in anxious patients. Finally, this study sought to measure endogenous cortisol and cortisone concentrations prior to, during, and after the CBT intervention, in order to determine their predictive value for clinical efficacy at one-month follow-up and change in fear bias on the day after treatment.

Methods: In a randomised, placebo-controlled, double-blind study, 33 healthy volunteers with high spider fear were randomised to receive a single dose of hydrocortisone (20mg) or placebo one hour prior to a brief course of computerised and therapist-led exposure-based CBT. Treatment outcome was evaluated using self-report clinical symptom questionnaires, a behavioural approach task, and an implicit evaluation task measuring fear bias. Cortisol and cortisone concentrations were

analysed from hair and saliva samples using liquid chromatography tandem mass spectrometry. Cognitive-bias measures were re-evaluated the day following treatment, and all clinical outcome measures were obtained at one-day and one-month follow-up.

Results: Compared to placebo, hydrocortisone did not augment the clinical efficacy of one-session CBT. Early changes in the fear bias on the day after treatment were not predictive of clinical symptomatic improvement one-month later in either the hydrocortisone or placebo group. Hydrocortisone administration resulted in a significant acute increase in salivary cortisol and cortisone. Salivary glucocorticoid measurements during treatment and on the morning of treatment did not predict clinical efficacy at one-month. Endogenous baseline hair cortisol did not predict clinical symptomatic improvement. There was a non-significant trend for lower baseline hair cortisone to predict enhanced one-month improvement in the drug group, but not the placebo group. Baseline hair cortisol was not predictive of early changes in the magnitude of fear bias whilst hair cortisone significantly predicted fear bias change across the groups. There was a non-significant trend for this relationship to be stronger in one of the groups, with lower hair cortisone predicting greater fear bias change in the 24 hour period after treatment in the hydrocortisone group, but not in the placebo group.

Conclusions: This study provides insight into the interaction between the endogenous glucocorticoid system, exogenous hydrocortisone administration, and the mechanisms and trajectories of clinical symptomatic recovery following a single session of exposure-based CBT. It also highlights the potential for baseline endogenous hair cortisone concentration to be used to distinguish individuals who are likely to respond to therapy from those who are not. An approved understanding of these phenomena, as well as an appreciation of the methodological limitations of this study, will likely be key to enhancing the armoury of psychological and pharmacological tools to more effectively treat anxiety disorders in the future.

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1. Introduction

1.1 Prevalence and pathophysiology of anxiety disorders

Fear represents an adaptive response to threat. It enables an individual to escape harm and thus from an evolutionary perspective, fear ultimately ensures survival and continuation of the species. However, in anxiety disorders, the fear response becomes grossly exaggerated. A previously neutral stimulus becomes paired with an aversive stimulus through classical conditioning. Encountering the stimulus generates feelings of hypervigilance and uncontrollability in the individual, who engages in avoidance and/or compulsive behaviours to alleviate the experienced distress. The ensuing reduction in anxiety negatively reinforces these behaviours, whilst simultaneously preventing new learning opportunities that could potentially extinguish the conditioned fear response. Together, these contribute to the maintenance and enhancement of the condition (McGuire et al. 2014).

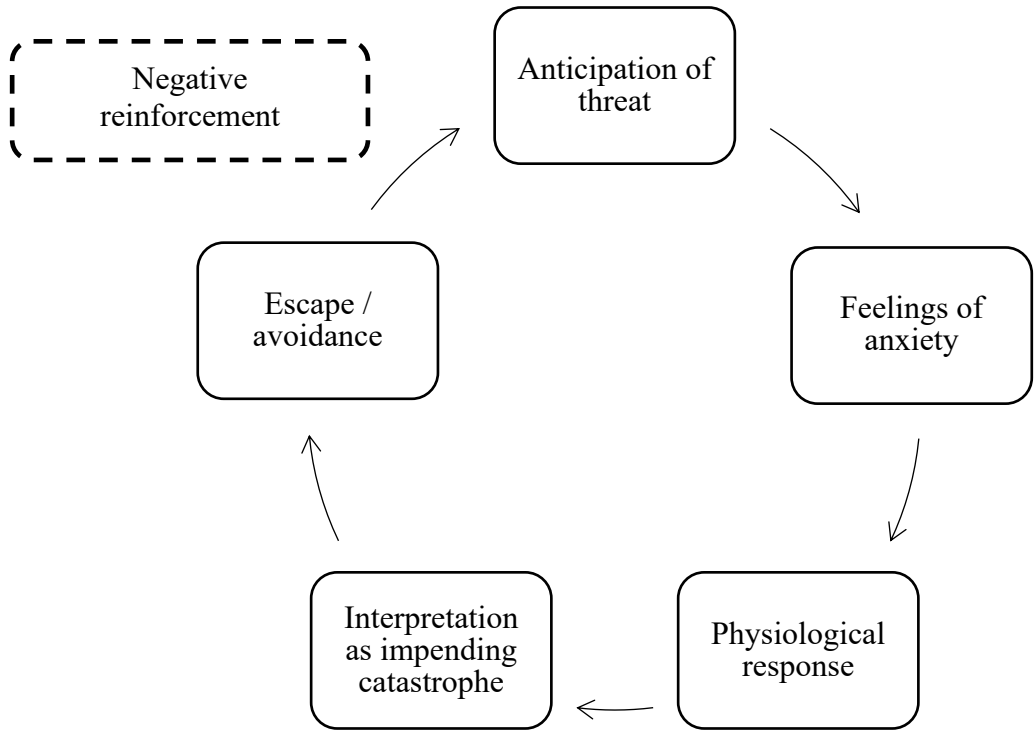

Figure 1. The anxiety cycle. The anxiety maintaining role of escape and avoidance in the genesis, perpetuation and enhancement of anxiety disorders.

With a lifetime prevalence of over 25%, anxiety disorders are the most common mental health condition (Kessler et al. 2005). Specific phobias represent the most prevalent type of anxiety disorder, with an estimated lifetime prevalence of 12.5% (Kessler et al. 2005; Michael et al. 2007). Specific phobias are characterised by an exaggerated fear response to a particular object, animal, place or situation. Exposure to the phobic stimulus leads to strong physiological and emotional manifestations of fear, in addition to cognitive symptoms and behavioural reactions, especially avoidance of the feared trigger (American Psychiatric Association 2013). Overall, anxiety disorders pose a particularly large economic burden (Koopmans et al. 2005) and can cause significant impact on individual quality of life and psychosocial functioning, including detrimental effects on relationships, parenting and career productivity (Greenberg et al. 1999; Leon et al. 1995; Mendlowicz & Stein 2000; Zaider et al. 2010).

1.2 Current treatment for anxiety disorders

The National Institute of Health and Clinical Excellence (NICE) currently recommend two first-line treatments for anxiety disorders. These are either (i) pharmacological interventions, typically selective serotonin reuptake inhibitors (SSRI) or serotonin-norepinephrine inhibitors (SNRI) or (ii) psychotherapeutic approaches, typically cognitive behavioural therapy (CBT) (National Institute of Health and Clinical Excellence [NICE] 2011). However, neither pharmacological nor psychotherapeutic approaches are without their flaws. Drug therapy is associated with long term side effects (Mavissakalian et al. 2002), whilst for the minority of patients who gain access to CBT treatment, courses can be long and cost-intensive (Gunter & Whittal 2010; Otto et al. 2000). Moreover, failure to treat a subgroup of patients and relapse following discontinuation are commonly associated with both pharmacological (Bruce et al. 2005; Davidson et al. 2004) and psychological therapies (Hans & Hiller 2013; Ali et al. 2017).

1.3 Exposure therapy

CBT for anxiety disorders is centred around exposure therapy, an approach which has proven effective in many individuals (Norton & Price 2007; Hofmann & Smits 2008; Öst 1989). Exposure therapy involves repeated interaction with the fear-provoking stimulus without engaging in compulsive or avoidance behaviours, until the patient's mal-adaptive fear response is reduced or extinguished. Corrective associations begin to form through the process of habituation as the subject learns that the catastrophic outcome they

envisaged happening will not occur if they remain in the presence of the fear-provoking stimulus (Kaczkurkin & Foa 2015). Thus, through repeated and graduated exposure, the negative reinforcement paradigm maintaining the anxiety disorder is extinguished and the severity of symptoms decrease. Despite its efficacy, several disadvantages to exposure-based CBT initiatives deserve mention. Critically, it has been shown that as many as 40-60% of patients do not adequately respond to treatment and that only a limited number of individuals achieve symptomatic remission (Walkup et al. 2008; Schnurr et al. 2007; Loerinc et al. 2015). Moreover, participant attrition (commonly due to difficulty with exposure, perceived lack of immediate symptomatic relief and treatment burden) poses an added challenge (Hofmann & Suvak 2006; Abramowitz 1997). Finally, relatively few therapists are trained to provide exposure-based CBT and as a result, for many patients, access to treatment is unattainable or incurs a long waiting period (McGuire et al. 2014).

1.4 Improving treatment

1.4.1 Computerised approaches

Given that a significant percentage of the population is affected by anxiety disorders yet current treatments remain sub-optimal, alternative approaches aiming to improve therapy have been developed. One such initiative is self-exposure treatment in which the patient adopts a more central role in the progression of their own therapy, whilst the need for clinical supervision is minimised. Self-exposure initiatives appear to be particularly effective in reducing anxiety and avoidance behaviour in specific phobias (Ghosh & Marks 1987; Mathews et al. 1977) for which they boast dropout rates comparable to those of therapist-directed treatment (Carr et al. 1988; Proudfoot et al. 2012). However, it appears that almost all (91%) anxious patients who are potential users of self-help therapies would require it to be computer-based, due to faster access to information and therapeutic help, combined with reduced fear of stigmatisation (Graham et al. 2001). The advantages of computerised CBT (CCBT) are manifold: it allows patients to access psychological therapies more quickly, can be delivered in a variety of settings, can regularly be updated, and can incorporate problem monitoring and feedback support. Further, being a patient-led treatment, CCBT can also promote agency, mastery, control and learned resourcefulness in the patient (Hands-on Help: Computer-aided Psychotherapy) (Liu et al. 2009). Overall, a meta-analysis of CCBT programs for anxiety disorders indicated a benefit over non-treatment comparison

groups and equivalent outcomes to face-to-face CBT therapies (Cuijpers et al. 2009). One particularly promising branch of CCBT, especially for specific phobias, is computer-based exposure therapy (CBE). The unique benefits of CBE initiatives have been demonstrated in a randomised control trial in 36 spider phobic individuals by Muller and colleagues. In contrast to the control group, patients receiving one-session of CBE therapy showed greater fear reduction from pre-treatment to post-treatment, an effect that remained stable at one-month follow-up (Müller et al. 2011).

1.4.2 Glucocorticoid administration

Another approach aimed at improving treatment for anxiety disorders has been to modulate the pathophysiological processes that maintain anxiety disorders using drugs. A promising candidate is the drug hydrocortisone, a functional analogue of the endogenous glucocorticoid hormone cortisol. In recent years we have begun to appreciate how glucocorticoids influence the neural networks that mediate learning and memory. Emerging evidence demonstrates that that glucocorticoids impair the learning and memory process that are highly involved in the genesis and maintenance of anxiety disorders (De Quervain et al. 2009). Specifically, glucocorticoid administration appears to impair memory retrieval (De Quervain et al. 2000; Het et al. 2005; Merz et al. 2010), with emotionally arousing memories being especially sensitive to these effects (Domes et al. 2004; Kuhlmann et al. 2005). Glucocorticoids have also been demonstrated to facilitate the consolidation of extinction memory, whereby suppression of glucocorticoid action impairs the extinction process (Bohus & Lissák 1968; Cai et al. 2006).

Given that glucocorticoids appear to impair memory retrieval but simultaneously promote the consolidation of extinction memories, recent research efforts have focused on whether this concept can be translated to improving anxiety disorder treatment. A clinical study investigating the effects of cortisol administration in patients with chronic post-traumatic stress disorder (PTSD) found evidence for a beneficial effect. Low-doses of cortisol (10mg per day) was administered orally each day for one month, and after this period patients reported reduced retrieval of intrusion symptoms, without any adverse side effects (Aerni et al. 2004). Similarly, two double-blind, placebo-controlled clinical studies led by Soravia and colleagues in 2006, demonstrated that glucocorticoid administration could reduce stimulus-induced fear in patients with social and spider phobia (Soravia et al. 2006). In the social phobia study, 40 subjects received a 25mg oral

dose of cortisone one hour prior to a socio-evaluative stressor. This led to reduced self-reported fear during the anticipation, exposure and recovery phases of the stressor compared to placebo. Moreover, in placebo-treated subjects, there was negative correlation between stress-induced cortisol release and fear ratings, suggesting that endogenously released cortisol may buffer fear symptoms in a phobic environment. In the spider phobia study, 20 subjects were repeatedly given 10mg of cortisol one hour before exposure to a spider photograph. Compared to placebo, cortisol treatment progressively reduced stimulus-induced fear during the session, and these positive effects remained two days later. Since it is impossible to directly measure the memory retrieval process in phobic disorders, it is possible that cortisol is simply reducing fear though direct anxiolytic effects in these studies, or by modulating other systems involved in fear expression. However, support to the notion that glucocorticoids reduce fear by inhibiting aversive memories is given by PTSD studies, in which reduced re-experiencing the trauma is a direct measure of memory retrieval (Aerni et al. 2004; Shelling et al. 2006). Furthermore, in the phobia studies (Soravia et al. 2006), glucocorticoid administration did not affect phobia-unrelated anxiety, suggesting that phobic memory structures were specifically affected.

In light of the evidence suggesting that glucocorticoid administration can reduce fear in patients with anxiety disorders, the theory emerged that it might be possible to enhance the efficacy of existing CBT techniques with exogenous glucocorticoid administration. A randomised, double-blind, placebo-controlled study by De Quervain et al. in 2011 provided compelling evidence that hydrocortisone significantly enhances the clinical effects of exposure-based CBT (De Quervain et al. 2011). 40 patients with specific phobia for heights were treated with three sessions of virtual reality exposure therapy. Cortisol (20mg) or placebo was given orally one hour before each treatment session. Results revealed that cortisol administration (i) reduced self-reported fear post-treatment and at one month follow-up, (ii) reduced acute anxiety during exposure to the phobic situation (post-treatment) and (iii) reduced exposure-induced increases in skin-conductance level at one-month follow-up, an effect previously shown to be associated with successful fear extinction (Vansteenwegen et al. 2007). Similarly positive results have been obtained by Soravia and colleagues in the context of spider phobia. 22 spider-phobic individuals received either cortisol (20mg) or placebo one hour before two *in vivo* exposure-based therapy sessions. When patients returned for follow-up one month later, it was found that

the cortisol group had a significantly greater reduction in spider fear compared to placebo (Soravia et al. 2014). Further evidence that cortisol administration can enhance the therapeutic gains of exposure therapy is evident in the context of PTSD, with two initial pilot studies revealing that exogenous glucocorticoid administration improved the clinical outcome and longevity of exposure based CBT (Surís et al. 2010; Yehuda et al. 2015). Interestingly, in one study, it was also observed that PTSD patients who benefitted the most from glucocorticoid-enhanced exposure therapy were those who possessed the highest pre-treatment glucocorticoid sensitivity (Yehuda et al. 2015), suggesting an interaction between the effects of exogenous and endogenous glucocorticoids on treatment outcome.

1.5 The future of treatment?

The above studies converge on the hypothesis that glucocorticoids might not only be involved in, but may also modulate the effects of extinction-based psychotherapeutic interventions in anxiety disorders. Given that computerised exposure therapy appears to be both effective and preferred by anxious patients, there is impetus to investigate whether glucocorticoid administration can enhance the efficacy of computerised approaches. This approach has yet to be tested and could lead to dramatic changes in the current treatment of anxiety disorders. However, in order to best enhance the efficacy of interventions, we must first acquire a deeper understanding of the basic mechanisms by which treatments exert their effects. Doing so will not only enable us to predict whether a patient is likely to benefit from a specific form of therapy prior to its cost- and labour-intensive application, but will also provide an improved platform for treatment augmentation and optimum combination of different treatment ingredients.

1.6 Endogenous glucocorticoids and anxiety disorders

The hypothalamic-pituitary-adrenal (HPA) axis and its end product cortisol are key players in mediating the body's response to stress (Cacioppo et al. 2016). Release of the endogenous glucocorticoid hormone cortisol from the adrenal cortex is pulsatile, resulting in a peak in the morning and low concentrations during the evening and night (Weitzman et al. 1971). Alterations to the HPA axis are a common pathophysiological finding in various anxiety disorders. Although results are far from consistent, panic disorder appears to be characterised by raised basal urinary cortisol levels, followed by a decline after successful pharamacological intervention. Patients with generalised anxiety

disorder typically exhibit a decline in cortisol levels following successful psychological or pharmacological treatment, however pre-treatment cortisol levels remain debated. On the other hand, specific phobia appears to be characterised by cortisol levels which rise during encounter of the feared stimulus (Elnazer & Baldwin 2014).

1.7 Cortisol as a marker of treatment outcome

A key limitation of current CBT techniques is their inefficacy in a sub-group of patients. One option that could maximise efficacy across the entire patient cohort would be to find pre-treatment features that distinguish patients who are likely to respond from those who are not. This would enable therapy to be tailored to the individual whilst simultaneously guiding the development of novel interventions. Currently, the most reliable predictor of non-response is the severity of the anxiety disorder and the co-morbidity of the anxiety disorder with other mental health conditions, particularly personality disorders. However, markers that better reflect the mechanisms underpinning the non-response phenomenon are desirable. One candidate marker is endogenous cortisol concentration.

There is evidence to suggest that endogenous cortisol levels during exposure therapy correlate with enhanced clinical results. For example, a small naturalistic study in 10 patients with panic disorder with agoraphobia revealed that greater absolute cortisol levels during flooding therapy were moderately but consistently related to improved therapy outcome (Siegmund et al. 2011). Building on this, a slightly larger study conducted by Meuret and colleagues in 26 patients with panic disorder and agoraphobia also provided evidence for higher cortisol levels during exposure being linked to improved psychological therapy response (Meuret et al. 2015). In line with these findings, Lass-Hennemann and Michael found that exposure therapy in spider phobic individuals was more effective in the morning than in the evening i.e. when endogenous cortisol levels were increased due to the circadian rhythmicity of cortisol output (Lass-Hennemann & Michael 2014).

However, compared to laboratory stress-induction, elevated and prolonged anticipatory anxiety is more reflective of *in-vivo* exposure, as subjects anticipate confronting distressing stimuli. Accordingly, it has been demonstrated that cortisol elevations can be detected one hour prior to exposure therapy in patients with phobia of driving (Alpers et al. 2003) and that salivary cortisol is increased prior to a stressor task in individuals with

social phobia (Condren et al. 2002). Furthermore, in the context of PTSD, evidence has also accumulated in support of the potential of pre-treatment glucocorticoid biomarkers to be used as predictors of successful psychotherapeutic outcome (Colvonen et al. 2017). Specifically, higher pre-treatment bedtime salivary cortisol levels and urinary glucocorticoid concentration predicted greater improvements in PTSD symptomatology post-treatment (Yehuda 2009; Yehuda et al. 2014). Further, a recent study highlighted that enhanced HPA axis negative feedback sensitivity before treatment was an important predictor of greater PTSD symptom decrease in response to trauma-focused psychotherapy (Nijdam et al. 2015). This finding is complemented by data suggesting that higher pre-treatment cortisol stress reactivity is related to greater reductions in PTSD symptomatology in response to CBT (Rauch et al. 2015).

There is also evidence indicating an interaction between morning cortisol rises, or the cortisol awakening response (CAR) and clinical outcome following psychotherapeutic treatment in individuals with anxiety disorders. The CAR has been demonstrated to be a reliable biological marker for HPA axis activity in children, adolescent and adults when measured repeatedly with strict reference to the time of awakening (Clow et al. 2004; Hellhammer et al. 2009). Meuret et al. found in their study in patients with panic disorder and agoraphobia that a greater CAR was associated with enhanced treatment outcome (Meuret et al. 2015). However, others have demonstrated conflicting results with two studies revealing that the CAR had no effect at all on the anxiety disorder treatment response (Brand et al. 2011; Dierckx et al. 2012).

As previously mentioned, a series of studies has revealed that exogenous glucocorticoid administration can enhance the efficacy of exposure-based therapies in patients with different kinds of phobias. (Soravia et al. 2006; De Quervain et al. 2011; Soravia et al. 2014). However, it is only more recently that research has alluded to the existence of an interaction effect between endogenous glucocorticoid functioning and exogenous glucocorticoid administration. For example in the context of PTSD, it has been observed that patients who benefitted the most from glucocorticoid-enhanced exposure therapy were those who possessed the highest pre-treatment endogenous glucocorticoid sensitivity (Yehuda et al. 2015).

However, the results of the aforementioned studies investigating endogenous glucocorticoid levels must be interpreted with caution. Almost all studies to date have used short-term cortisol markers including urinary, salivary or plasma cortisol, thus cannot inform about long-term cortisol secretion. This is surprising given that most anxiety disorders are characterised by long-term HPA axis disturbances (Elnazer & Baldwin 2014). Furthermore, many studies have only assessed the cortisol awakening response which represents a distinct aspect of diurnal cortisol secretion and poorly reflects overall cortisol output.

1.8 Markers of treatment success – the 'fear bias'

A key factor hindering the amelioration of current treatments for anxiety disorders is the fact that markers of diagnosis and treatment efficacy have proven difficult to identify. However, an emerging theory is that biased processing of negative information (a 'fear bias') may represent a surrogate marker for the aetiology and maintenance of anxiety disorders, as well as for the efficacy of interventional approaches (Reinecke, Waldenmaier, et al. 2013; Mathews & MacLeod 2005). There is extensive evidence that relative to non-anxious controls, highly anxious individuals possess i) faster response latencies and detection of negative material (e.g. dot probe task, exogenous cuing, attentional blink, emotional stroop), ii) difficulties in disengaging from threatening stimuli, in relation to neutral or positive stimuli and iii) the tendency to interpret ambiguous scenarios in a negative manner (Fernández et al. 2017). Both CBT and pharmacological interventions have been shown to increase the threshold for processing negative information (Mogg et al. 1995; Teachman et al. 2008; Reinecke, Rinck, et al. 2013), suggesting that normalisation of the fear bias might be key to recovery in anxious patients. A particularly critical finding has been that alterations to the fear bias are measurable *before* symptomatic change is subjectively experienced, and that these early changes in bias correlate with the magnitude of subsequent clinical improvement. This suggests that alterations to fear biases are a mediator of symptomatic change, rather than simply a marker of clinical outcome (Reinecke, Waldenmaier, et al. 2013).

In contrast, it has traditionally been assumed that CBT targets explicit and deliberate cognitive beliefs, rather than automatic processes (Harmer et al. 2009; Derubeis & Hollon 2009; Gorman et al. 2000). For example, in the one-session therapy (OST) for specific

phobias, patients are encouraged to recognise and challenge explicit cognitions including catastrophising thoughts such as "the spider will jump on me" or "I will not cope" (Öst 1989). Symptomatic relief is theorised to occur over time through repeated learning and practice. However, a paradigm shift in our understanding of the mechanisms of therapy are challenging this concept. Studies have revealed that biases in automatic attention and *implicit* fear evaluations in memory, but not biases in *explicit* memory, are present in patients with anxiety disorders (Lebens et al. 2011; Mathews & MacLeod 2005). Moreover, it has been also demonstrated that implicit fear evaluations are sensitive to CBT in specific phobia (Reinecke et al. 2012; Teachman & Woody 2003), generalised anxiety disorder (Reinecke, Rinck, et al. 2013) and panic disorder (Teachman et al. 2008).

1.9 Glucocorticoids and attentional bias

Considering that normalising the fear bias appears to be prerequisite for clinical symptomatic changes during CBT for anxiety disorders (Reinecke et al. 2012; Reinecke, Waldenmaier, et al. 2013; Reinecke, Rinck, et al. 2013), the possibility arises that cortisol-augmented CBT outcomes (De Quervain et al. 2011) also depend on this process.

Van Honk et al. found that in a sample of 28 students, higher baseline cortisol levels were associated with lower attentional bias towards angry faces for masked (subliminal) stimuli (Van Honk et al. 1998). They later translated this finding to unmasked stimuli in a similar study in 40 male students (Van Honk et al. 2000). An additional finding was that in subjects who demonstrated attentional bias to both masked and unmasked stimuli, cortisol levels significantly increased following presentation of the threatening stimuli, likely because attending the threatening stimuli activated the cortisol stress response. A more recent study in 20 young men revealed that exogenous cortisol administration reduced attentional bias towards masked fearful faces only in individuals with elevated anxiety (Putman et al. 2007). Interestingly, in a similar study in healthy, non-anxious subjects using unmasked presentations, an association between cortisol administration and attentional bias could not be found (Vasa et al. 2009).

Overall, identifying additional components that could improve computerised CBT initiatives has the potential to incur a drastic paradigm shift in the treatment of anxiety disorders. A promising candidate to elicit such effects is the drug hydrocortisone, which

may augment therapeutic gains through interaction with the endogenous glucocorticoid system and manipulation of the fear bias interface.

2. This study

2.1 Aims and objectives

The present study aimed to harness the untapped potential of glucocorticoids to augment the efficacy of one-session computer-based exposure therapy in a sample of spider fearful individuals. The rationale behind this interventional study is to provide a rapid, practical and cost-effective treatment for anxiety disorders, building on the previous success of brief computer-based exposure initiatives (Müller et al. 2011). The primary objective of this study is to examine whether hydrocortisone administration can enhance the clinical effectiveness of one-session CBT. As a secondary objective, this work will examine whether the benefits of hydrocortisone-augmented exposure therapy are driven through modification of the fear bias. Finally, this study will explore the relationships between endogenous cortisol and cortisone levels and exogenous hydrocortisone administration, and how they link to the clinical efficacy of one-session CBT.

2.2 Methodology

In a randomised, placebo-controlled, double-blind study design, 33 healthy volunteers with high spider fear were randomised to receive a single dose of hydrocortisone (20mg) or placebo. After one hour, when plasma drug levels were hypothesised to peak, participants engaged in a brief course of computerised and therapist-led exposure-based CBT. Treatment outcome was evaluated using self-report clinical symptom questionnaires, a behavioural approach task and an implicit evaluation task, measuring fear bias. Cortisol and cortisone concentrations from saliva and hair samples were measured before, during and after the CBT intervention. Outcome measures were monitored at one-month follow-up in addition to the day after treatment, enabling more reliable conclusions to be drawn regarding the stability of treatment effects.

2.3 Participant cohort

There were several reasons why we decided to test a cohort of individuals with high spider fear. Primarily, we have pursued spider phobia as a proxy anxiety disorder,

particularly because implicit evaluation tasks such as the Extrinsic Affective Simon Task (EAST) can effectively be developed for specific phobias where the feared stimulus is easily identified and reproduced for treatment. Additionally, spider phobia represents a considerable disease burden in its own right with a lifetime prevalence of 2.7–5%. (Oosterink et al. 2009; Margraf et al. 2000), thus enhancing treatment would improve quality of life for a significant number of individuals. Given that specific phobias represent an accurate model of anxiety disorders in general, particularly with respect to their aetiology and maintenance, we hope that the results of this study can be extrapolated to other anxiety disorders, although this remains to be investigated.

2.4 Ethics

This study was in accordance with the *Ethical Principles for Medical Research Involving Human Subjects* formulated in the Declaration of Helsinki. The general study protocol was approved by the National Health Service research ethics committee of the University of Oxford. Written, informed consent concerning the specific procedure described herein was obtained from all participants before commencing the study. Subjects were informed that participation was entirely voluntary and that they could withdraw from the study at any time. Participants were monitored closely by a physician after drug intake, and if judged to be at any risk, or if they did not wish to continue, they would be withdrawn from the study. The study was conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996. The study protocol, informed consent form, participant information sheet and any proposed advertising material were submitted to the Medical IDREC and host institution for written approval. No amendments to the original approved documents were made throughout the study.

3. Hypotheses

The following hypotheses were tested. Each hypothesis is in bold, presented above the studies providing the rationale from which each statement is derived.

(i) Compared with placebo, hydrocortisone administration enhances the clinical efficacy of one-session, computer-based CBT at one-day and one-month follow-up.

The benefits of administering glucocorticoids prior to multiple exposure-based CBT sessions in patients with various phobias (Soravia et al. 2006; De Quervain et al. 2011; Soravia et al. 2014). We aim to translate these findings to a single session of computer-based CBT.

(ii) Hydrocortisone-enhanced CBT outcomes are driven by stronger early corrections in the fear bias on the day after treatment.

The fear bias may represent a surrogate marker for the aetiology and maintenance of anxiety disorders, as well as for the efficacy of interventional approaches (Reinecke, Waldenmaier, et al. 2013; Mathews & MacLeod 2005).

(iii) Hydrocortisone administration will result in an acute increase in salivary cortisol and cortisone.

Compared to placebo, hydrocortisone administration led to significantly higher salivary cortisol levels between 60 and 150 minutes after capsule intake (Soravia et al. 2014).

(iv) Greater salivary cortisol and cortisone levels during treatment predict enhanced clinical efficacy of one-session, computer-based CBT at one-month follow-up.

Greater absolute cortisol levels during psychotherapeutic treatment for anxiety disorders correlate with enhanced clinical outcome (Siegmund et al. 2011; Meuret et al. 2015; Gaab et al. 2005; Lass-Hennemann & Michael 2014).

(v) Greater morning rises in salivary cortisol and cortisone levels predict the clinical efficacy of CBT at one-month follow-up.

Rises in cortisol levels on the morning of psychotherapeutic interventional therapy are predictive of therapeutic outcome in patients with panic disorder and agoraphobia (Meuret et al. 2015).

(vi) Baseline endogenous hair cortisol and cortisone concentrations predict the clinical efficacy of CBT at one-day and one-month follow-up.

Hair analysis provides a reliable, retrospective indicator of cumulative, long-term cortisol and cortisone secretion over a period of several months (Russell et al. 2012; Stalder et al. 2012).

(vii) Baseline endogenous hair cortisol and cortisone concentrations predict the magnitude of change in fear bias at one-day follow-up.

Higher endogenous baseline cortisol levels are associated with lower attentional bias towards angry faces in healthy subjects (Van Honk et al. 1998; Van Honk et al. 2000).

4. Study implications

The results obtained from this study will deepen our understanding of how the glucocorticoid system modulates the efficacy of one-session CBT for anxiety disorders through manipulation of the fear bias. It will also provide insight into the relationship between endogenous glucocorticoid functioning, exogenous hydrocortisone administration and anxiety disorder treatment outcome. An approved appreciation of these phenomena will be key to enhancing the armoury of psychological and pharmacological tools available for more effective and compact treatment of anxiety disorders. Using a cohort of spider fearful individuals, we hope that the results of this study have the potential to be extrapolated to the treatment of other anxiety disorders, although this remains to be verified by future work.

Chapter 2 – The effect of hydrocortisone administration on fear bias and fear extinction following one-session CBT

5. Introduction

Despite the prevalent and often disabling nature of anxiety disorders, many patients receive no or inadequate treatment (Wang et al. 2000; Wang et al. 2005; Kessler et al. 2005). The current first line interventions as recommended by NICE are either (i) pharmacological, typically SSRI or SNRI drugs or (ii) psychotherapeutic, typically cognitive behavioural therapy (CBT) (National Institute of Health and Clinical Excellence [NICE] 2011). However, both approaches are riddled with disadvantages, notably high relapse rates and inefficacy in a subgroup of patients (Bruce et al. 2005; Davidson et al. 2004; Hans & Hiller 2013; Ali et al. 2017).

The shortcomings of current approaches provide impetus to ameliorate their efficacy, or to combine various treatment ingredients to enhance therapeutic gains. One particularly promising approach has been the development of brief computer-based exposure (CBE) therapy, which appears to be effective not only post-treatment but also at one-month follow-up (Müller et al. 2011). An additional avenue of exploration has been to enhance the efficacy of traditional CBT initiatives with exogenous administration of glucocorticoids. This line of enquiry has proved effective in patients with a specific fear of heights (De Quervain et al. 2011) and in subjects with PTSD (Surís et al. 2010; Yehuda et al. 2015).

In recent years, a theory has emerged that normalisation of a perturbed fear bias is prerequisite for the symptomatic changes seen during CBT treatment for mood disorders (Reinecke et al. 2012; Reinecke, Waldenmaier, et al. 2013; Reinecke, Rinck, et al. 2013). Prior to this, it had been assumed that CBT primarily targets explicit and deliberate cognitive beliefs rather than automatic processes (Derubeis & Hollon 2009; Barlow et al. 2000). Therefore, the question arises whether cortisol-augmented outcomes are also effectuated through modulation of the fear bias. Indeed, the association between endogenous cortisol levels and attentional bias are well documented (Van Honk et al.

2000; Van Honk et al. 1998), as is the effect of exogenous cortisol administration on attentional bias in those with elevated levels of anxiety (Putman et al. 2007).

Given that the fear bias represents not only a crucial marker, but also a mediator of symptomatic improvement in anxious patients (Reinecke, Waldenmaier, et al. 2013), interventions that target this interface possess the scope to vastly enhance therapeutic gains. One promising candidate to elicit these effects could be the drug hydrocortisone.

The present study therefore aimed to investigate for the first time the potential of hydrocortisone to augment the efficacy of one-session CBT therapy in participants with high spider fear. In addition, we investigated whether hydrocortisone administration altered the fear bias in anxious individuals, and whether this correlated with improved clinical outcome. Changes in treatment outcome were evaluated the following day and at one-month follow-up using self-report clinical symptom questionnaires (the Spider Anxiety Screening; SAS, and the Fear of Spiders Questionnaire; FSQ), a behavioural approach task (BAT) and an implicit evaluation task (the Extrinsic Affective Simon Task; EAST). By studying spider phobia as a proxy anxiety disorder, we hope that the results of this study can be extrapolated to the treatment of other anxiety disorders, although this remains to be tested.

6. Hypotheses

In this chapter, the following hypotheses were tested: The hypothesis is in bold, presented above the studies providing rationale from which each statement is derived.

(i) Compared with placebo, hydrocortisone administration enhances the clinical efficacy of one-session, computer-based CBT at one-day and one-month follow-up.

The benefits of administering glucocorticoids prior to multiple exposure-based CBT sessions in patients with various phobias (Soravia et al. 2006; De Quervain et al. 2011; Soravia et al. 2014). We aim to translate these findings to a single session of computer-based CBT.

(ii) Hydrocortisone-enhanced CBT outcomes are driven by stronger early corrections in the fear bias on the day after treatment.

The fear bias may represent a surrogate marker for the aetiology and maintenance of anxiety disorders, as well as for the efficacy of interventional approaches (Reinecke, Waldenmaier, et al. 2013; Mathews & MacLeod 2005).

7. Method

7.1 Participants

In this randomised, double-blind, placebo-controlled study, participants were tested at the Department of Psychiatry at the University of Oxford. 33 healthy, spider-fearful males and females from the general public were recruited using local media (websites and newsletters) and posters and flyers in universities and other public spaces. Individuals interested in the study were sent a participant information sheet, and screened for the following inclusion criteria: fluency in English, 18-60 years old, light or non-smoker (to prevent cravings during testing), body mass index (BMI) of 18-30 kg/m^2 and a score of 14 or higher on the first three questions of the Spider Anxiety Screening (SAS; (Rinck et al. 2002)) at pre-screening and baseline (Appendix A). Subjects were excluded if they fulfilled any of the following conditions: pregnancy or breast-feeding; current use of hydrocortisone-containing medication; use of anti-depressant or anxiolytic medications

such as SSRI or SNRI during the last six weeks; lifetime history of bipolar disorder or psychosis, alcohol, medication or drug abuse or dependence; a current primary depressive disorder or first-degree family member with a history of psychosis or bipolar disorder; severe insufficiency of the kidneys; lifetime history of serious physical illness (as determined by the study medical doctor); previous psychological treatment for spider fear and inadequate English skills.

7.2 Study Design

Subsequent to assessment of the above inclusion and exclusion criteria via email or phone conversation, subjects were invited for a further initial screening session. Following informed written consent, participants were screened for any potential axis-I psychological disorders using the Structured Clinical Interview for DSM-V-TR Axis I Disorders (SCID, 21; (First et al. 2002)) and underwent a brief basic physical examination performed by the medical doctor to ensure they were generally healthy to take hydrocortisone. In line with previous work, it was also confirmed if the participant fulfilled all DSM-V criteria for a specific phobia. Participants not fulfilling the 'impairment of functioning' criterion were included as well, considering that avoiding confrontation with spiders is relatively easy in Western Europe. If the screening visit qualified the participants as eligible, they were recruited into the study and invited for testing visits.

Prior to study commencement, a randomisation sheet was drawn up by a researcher without direct contact with study participants. Blocked randomisation was used whereby patients were allocated to sequential numbers whilst stratifying for gender (F = female/M = male). Study treatment (hydrocortisone versus placebo) was allocated to these sequential numbers randomly in blocks of four per stratification combination. For example, out of the first four female participants, two were given placebo, and two received hydrocortisone. The order of treatments in these blocks of four was generated using an online tool for randomisation (www.random.org). Participant numbers were given to patients sequentially as they entered the study, and appropriate medication was provided accordingly (based on running number and gender). Both trial participants and the investigator were blinded to the nature of the study medication dispensed. Of the total sample of 33 participants, 16 received hydrocortisone and 17 received placebo.

7.3 Procedure

Following the screening visit, each participant attended three study visits in total. The first testing visit took place no later than one month after the screening visit and women who were not taking any form of oral contraceptive were tested during the premenstrual week. All participants were asked to fast for two hours prior to the appointment, to ensure homogenous effects of hydrocortisone across subjects. During the first testing visit, participants completed a battery of psychological and cognitive questionnaires including: the Attentional Control Scale (ACS, Derryberry & Reed 2002), the Anxiety Sensitivity Index (ASI, Taylor 1998), the Beck Depression Inventory (BDI, Beck et al. 1996), the Behavioural Inhibition Scale (BIS, Carver & White 1994), the Behavioural Activation Scale (BAS, Carver & White 1994), Eysenck Personality Questionnaires (EPQ, Eysenck & Eysenck 1994), the Perceived Stress Reactivity Scale (PSRS, Schlotz et al. 2011), the State-Trait Anxiety Inventory (STAI, Spielberger 1983) and a trauma history checklist (THC, Holmes et al. 2004) (Appendix B). Baseline clinical assessments were carried out using self-report clinical symptom questionnaires (Spider Anxiety Screening; SAS, Fear of Spiders Questionnaire; FSQ, Appendix C), a behavioural approach task (BAT) and an implicit evaluation task (EAST). Subsequently, participants received a single dose of hydrocortisone or placebo. Capsule administration took place between 1pm and 4pm since circadian rhythmicity is known to affect HPA axis (Weitzman et al., 1971). When peak plasma levels were reached, approximately one hour after capsule intake, participants engaged in a single-session of computerised and therapist-led CBT. At baseline and at peak drug level, physiological parameters (heart rate and blood pressure) were tested, as was mood using visual analogue scales (Appendix D), to determine whether hydrocortisone had any effect on these variables. At the end of the testing session, participants and therapists completed a demand questionnaire asking them to guess whether they had received hydrocortisone or placebo and to provide a reason for their answer. The following day and after one month, participants returned to be assessed on all clinical (SAS, FSQ, BAT) and cognitive bias (EAST) outcome measures. After the final session, participants were debriefed and compensated for their time (£30).

7.4 Clinical symptom measures

Spider anxiety was measured using the FSQ (Szymanski & O'Donohue 1995) and the SAS (Rinck et al. 2002). These were used at screening, testing, one-day and one-month follow-up. The SAS is a short, 4-item questionnaire concerning spider fear, avoidance,

physiological manifestations of spider-related anxiety and the burden of the fear. These four items are rated on a 0-6 Likert scale. The SAS shows very good test-retest reliability (r = 0.89, Reinecke et al. 2010) and was used here for screening purposes and as a primary outcome measure. All four items were used to compute a total score that measured changes from baseline to one-day and one-month follow-up. The FSQ is an 18-item self-report questionnaire addressing physiological, cognitive, behavioural and emotional reactions to spiders, rated on a 0-6 Likert scale. The FSQ has been shown to discriminate spider-phobic individuals from controls (Szymanski & O'Donohue 1995) and to be responsive to treatment (Muris & Merckelbach 1996). It has also demonstrated good test-retest validity (r = 0.95, Reinecke et al. 2010) and internal validity (Cronbach's alpha = 0.69, Rinck et al. 2002).

7.5 Fear bias measure

Fear bias was assessed using the EAST (De Houwer 2003), with fear-related stimuli that have been used in previous research (Reinecke et al. 2010; Reinecke et al. 2012). The EAST demonstrates sufficient reliability (1-week test–retest reliability: r = 0.42, $p < 0.001$; internal consistency: Cronbach's $\alpha = 0.44$) and validity (correlation with FSQ: r = −0.32, $p < 0.01$) (Reinecke et al. 2010). The task comprises two stages: a practice stage consisting of a valence practice block and a target practice block, and an experimental stage consisting of five experimental blocks. In the valence practice block, participants learnt to associate particular computer keys with positive or negative associations (valency) by using keys on the keyboard to categorise words with obvious positive or negative meanings, such as "fear" or "beautiful". In the target practice block, the same keys were used to categorise pictures of dragonflies (and their mirror images) based on the direction they were facing. Participants were asked to press one key if the dragonfly faced left and another if the dragonfly faced right. Having learned to associate keys with a particular valency and a particular gaze direction, five experimental blocks each comprising 40 images were delivered in a pseudo-randomised order. Stimuli were five spider and five butterfly pictures facing in different directions, and a total of 40 target words. Butterfly images were included as controls of no interest. The mixture of stimuli meant that participants sometimes had to respond to images of spiders using the key with positive valency (incongruent trials) and sometimes using the key with negative valency (congruent trials). The EAST measures reaction time for each trial. Because of cognitive dissonance, in the presence of fear bias, reactions are assumed to be faster if the

association of the image (e.g. negative for the picture of a spider) is the same as the association for the key (e.g. “unpleasant key”). This allows computation of the extent of the fear bias by subtracting reaction times for incongruent trials from reaction times for congruent trials for the spider pictures.

7.6 Behavioural approach task (BAT)

A behavioural outcome measure was used at three time-points: baseline (first testing visit prior to drug intake or CBT), one-day follow-up and one-month follow-up. In this task, participants were encouraged to walk along a corridor toward a transparent plastic terrarium with a lid, six metres away, containing a single tarantula carapace, which appeared to be a live spider. Carapaces, rather than live spiders were used to reduce the burden of caring for live spiders, whilst retaining realism. They were asked to approach the terrarium as quickly and closely as they felt able. The final proximity to the terrarium and the time taken to approach were measured, allowing approach speed to be calculated. The therapist did not provide any support or encouragement to the participant, other than detailing to them the initial task instructions.

7.7 Exposure-based CBT

45-minute CBT involved a combination of psychoeducation, CBE to spider stimuli validated in previous research (Müller et al. 2011), and brief therapist-guided exposure to a dead spider. CBT was deliberately kept brief in order to reduce the likelihood of the ceiling effect, which may have precluded further treatment enhancement with hydrocortisone. Psychoeducation first involved presenting the participant with written information about the anxiety response, before explaining to the ‘fight or flight’ reaction and the anxiety-maintaining role of avoidance. Participants were asked to summarise the information in their own words to check their understanding. Standard CBT techniques based on Öst (Öst 1989) were used to help participants identify negative automatic thoughts, perform basic thought challenging, and understand the need for behavioural experiments based on reducing avoidance. Training and supervision were provided by an experienced clinical psychologist, who supervised all diagnostic assessments and therapy.

The computerised exposure treatment was a 27-minute protocol that has previously been shown to be effective in reducing spider fear on self-report measures and a BAT in a

randomised controlled trial (Müller et al. 2011). The exposure protocol involved displaying nine medium-sized, fear-arousing spider images for three minutes each on the computer screen. To ensure that participants attended all parts of the stimulus and in order to reduce avoidance, they were instructed to click on a series of stars which were placed on various parts of the spiders' bodies and told that their responses were monitored. Therapists also encouraged participants not to look away from the screen whilst the images were presented. A practice trial with a non-spider image (a cup) was provided at the start to familiarise participants with the task.

In the last stage of treatment, participants were given the choice to engage in a final challenge. This involved approaching a sealed, transparent petri-dish containing a medium-sized dead spider. All participants chose to engage in the task. Participants completed visual analogue scales of distress before, during and after the task, and were encouraged to approach and handle the petri dish until distress levels had dropped by at least 50%. The therapist encouraged participants to challenge negative automatic thoughts during this time.

7.8 Medication

Participants were randomly allocated to receive a single dose of hydrocortisone (20mg; Teva UK Limited) or placebo, taken orally in capsule form. The major ingredient of the placebo capsules (Rayotabs Milchzuckertabletten, Rayonex GmbH) is microcrystalline cellulose. To make sure that placebo and active drug were non-identifiable by the participant, original hydrocortisone and placebo tablets were over-encapsulated in capsules of identical size and colour. Single-dose capsules were packed in non-transparent containers and taken by the participant in the presence of the researcher.

7.9 Statistical analysis

Statistical analyses were performed using SPSS for Windows, version 22 (IBM, Chicago, Illinois) at the Department of Psychiatry, Oxford University. Independent samples t-tests and chi-squared tests were used to determine any differences in baseline socio-demographic, psychological and cognitive characteristics in the hydrocortisone group compared to the placebo group. Mixed-model ANOVAs were run for each visual analogue scale (VAS) item, heart rate and blood pressure, with the within-subjects factor

time (baseline, drug peak) and the between-subjects factor group to identify any potential side effects following hydrocortisone administration compared to placebo. To further explore the nature of any interactions arising in this step, independent t-tests were used to determine at which timepoint(s) the hydrocortisone and placebo group scores significantly differed. To test the hypothesis that hydrocortisone administration enhances the clinical efficacy of one-session CBT at one-day and one-month follow-up, mixed-model ANOVAs were used to analyse each clinical outcome measure. Time (baseline, next day and one month) was used as the within-subjects condition and group was used as the between-subjects factor. In order to evaluate the effect of treatment on cognitive bias measures, attention bias scores were computed for the EAST and only correct spider trials were included in the analysis. Mixed ANOVAs were used to explore change over time (baseline and next day) between the hydrocortisone and placebo groups. To test the hypothesis that early changes in the fear bias on the day after treatment are predictive of enhanced clinical outcome at one-month, mediation analyses (multiple linear regression) as per Kraemer et al. were conducted, controlling for initial baseline clinical severity (Kraemer et al. 2002).

8. Results

8.1 Baseline characteristics

Table 1 illustrates that participants in the hydrocortisone and placebo groups were well-matched regarding their socio-demographic, psychological and cognitive characteristics reported at baseline ($p > 0.11$).

	Placebo group (N = 17)	**Hydrocortisone group (N = 16)**	***p* value**
Socio-demographics			
Females (%)	76.5	81.3	0.75
Age, years	24.1 (6.1)	25.3 (8.2)	0.64
Education, years	17.8 (2.9)	17.3 (2.7)	0.65
Specific phobia (%)	35.3	25.0	0.54
Psychological and cognitive characteristics			
ACS_total	54.3 (9.4)	52.7 (10.4)	0.65
ACS_focussing	23.4 (5.2)	23.1 (3.8	0.83
ACS_shifting	30.9 (6.1)	30.9 (5.0)	1.00
ASI	26.7 (14.3)	20.9 (15.6)	0.27
BDI	3.9 (4.8)	1.7 (2.4)	0.11
BIS_total	13.3 (3.4)	15.3 (3.6)	0.11
BAS_total	24.7 (4.1)	24.4 (3.9)	0.85
PSRS_total	20.4 (6.7)	17.3 (6.0)	0.18
STAI	37.8 (8.2)	33.8 (7.1)	0.14
EPQ_N	8.8 (4.7)	7.0 (4.3)	0.27
EPQ_P	3.2 (2.7)	2.8 (1.8)	0.60
EPQ_L	8.1 (4.2)	10.5 (4.6)	0.13
EPQ_E	13.8 (4.5)	14.0 (4.4)	0.88
THC	0.5 (0.9)	0.8 (1.3)	0.58

Table 1. Baseline socio-demographic, psychological and cognitive characteristics of participants in the hydrocortisone and placebo group (M, [SD] independent samples t-test/ Chi-squared test, *p*). ACS, attentional control scale; ASI, anxiety sensitivity index; BDI, Beck depression inventory; BIS, behavioural inhibition scale; BAS, behavioural activation scale; EPQ_N, Eysenck personality questionnaire_neuroticism; EPQ_P, Eysenck personality questionnaire_psychoticism; EPQ_L, Eysenck personality questionnaire_lie; EPQ_E, Eysenck

personality questionnaire_extraversion; PSRS, perceived stress reactivity scale; STAI, state-trait anxiety inventory; THC, trauma history checklist.

8.2 Testing for potential side effects of the drug

Mixed-model ANOVAs were run for each VAS item, heart rate and blood pressure, with the within-subjects factor time (baseline, peak) and between-subjects factor group. Participants in the hydrocortisone and placebo groups generally did not show any significant differences in changes in side effects from baseline to drug peak level (most $p \geq 0.06$, Table 2). However, the ANOVA indicated a significant group x time interaction for heart rate ($p = 0.04$). Follow-up independent two-sided t-tests comparing the two groups at baseline and drug peak level revealed no significant group difference at baseline ($p = 0.47$). The group difference at drug peak level was greater in participants who had taken hydrocortisone, although this group difference did not reach the conventional level of significance ($p = 0.22$, Figure 2). A significant time x group interaction also emerged from the mixed ANOVA for self-reported alertness ($p = 0.04$) and systolic blood pressure measurements ($p = 0.03$). Follow-up independent samples t-tests comparing VAS-recorded alertness at each time point revealed no significant differences at baseline ($p = 0.59$) or drug peak ($p = 0.07$) between the two groups. Follow-up independent samples t-tests also indicated no group differences at baseline for systolic blood pressure measurements ($p = 0.35$). Although systolic blood pressure appeared to decrease to a greater extend in the hydrocortisone group from baseline to drug peak level compared to the placebo group, this difference did not reach the level of significance ($p = 0.34$).

	Placebo N = 17		Hydrocortisone N = 16	
	M	**SD**	**M**	**SD**
BASELINE				
Physiological measures				
Heart rate	71.9	12.7	75.1	13.0
Blood pressure – systolic	119.5	14.4	124.5	15.9
Blood pressure – diastolic	74.6	9.1	76.2	8.8
VAS ratings				
Anxious	23.8	17.0	14.9	12.8
Sleepy	29.7	23.3	29.9	23.2
Flushed	10.5	15.2	17.1	20.6
Tearful	4.4	6.6	2.9	7.6
Nauseous	4.2	7.3	0.8	2.2
Hopeless	5.0	7.4	0.8	2.2
Tremor	2.9	4.2	3.1	6.5
Sad	5.6	7.3	2.1	4.2
Dizzy	4.5	7.4	2.1	5.8
Depressed	5.4	7.1	2.6	9.2
Tachycardic	8.7	9.2	3.0	6.9
Alert	47.2	19.5	51.3	23.3
DRUG PEAK				
Physiological measures				
Heart rate	69.3	10.7	64.8	10.1
Blood pressure – systolic	120.6	14.9	116.1	11.3
Blood pressure – diastolic	74.9	5.9	74.3	9.2
VAS ratings				
Anxious	15.5	13.5	19.9	21.8
Sleepy	36.1	23.6	24.9	22.0
Flushed	6.4	9.9	4.2	6.7
Tearful	5.1	6.5	2.6	5.5
Nauseous	4.6	6.4	2.3	4.5
Hopeless	4.5	6.0	0.9	2.5
Tremor	5.9	7.1	2.9	7.4
Sad	5.4	6.9	1.3	2.9
Dizzy	7.1	10.0	1.8	3.8
Depressed	4.3	4.4	1.4	3.1
Tachycardic	5.4	5.5	1.9	5.6
Alert	32.8	21.4	47.8	23.6

Table 2. Physiological measures and visual analogue scale (VAS) ratings in the two groups before drug intake and at drug peak level. M, mean; SD, standard deviation.

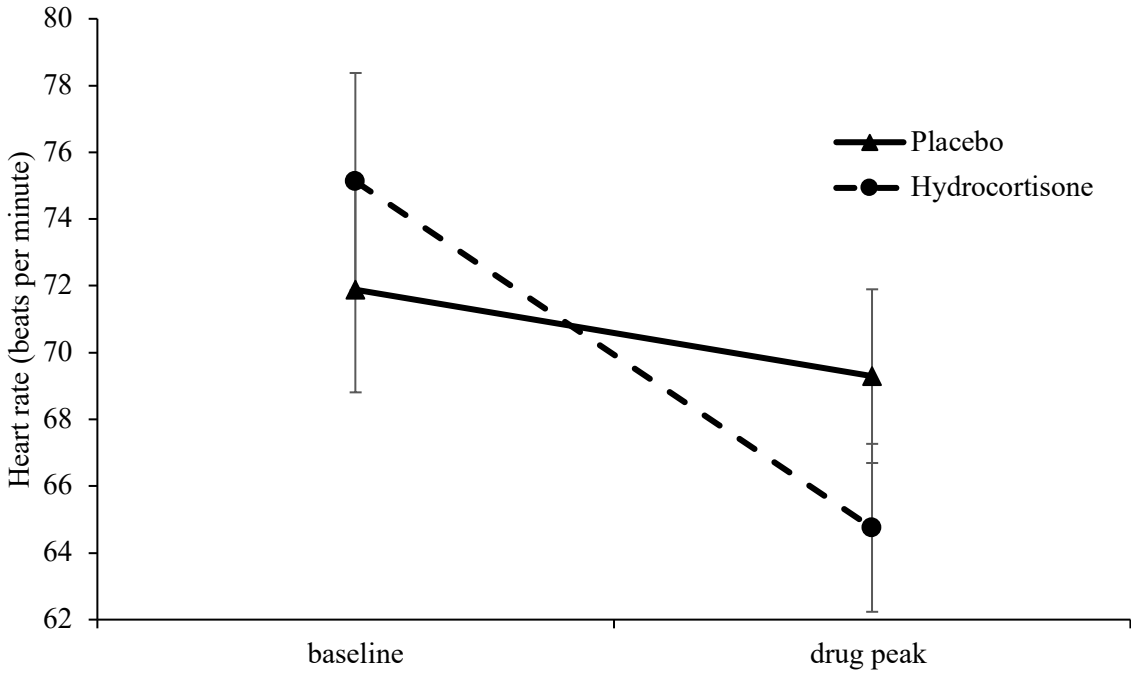

Figure 2. Heart rate in the two groups at baseline (before drug intake) and at drug peak level. Greater decreases in heart rate are evident in the hydrocortisone group, but the group difference at baseline and drug peak level did not reach the conventional level of significance. Error bars indicate standard error of the mean.

8.3 Demand questionnaires

Subjects in the two groups did not differ in how likely they were to report that they had been given hydrocortisone (placebo group: 3/17; hydrocortisone group: 4/16, chi-squared $p = 0.46$). Similarly, there was no indication that the experimenter was able to distinguish between participants who had received hydrocortisone and those who had not (hydrocortisone allocations experimenter: placebo group: 6/17; hydrocortisone group: 8/16, chi-squared $p = 0.31$).

8.4 Clinical symptom measures

All clinical outcome measures were analysed using mixed ANOVAs. Time was entered as the within-subjects factor and group as the between-subjects condition. Scores on the SAS decreased significantly over time, but without any significant differences between the hydrocortisone and placebo groups, main effect time $F(2,54) = 32.11$, $p < 0.001$; group x time $F(2,54) = 1.50$, $p = 0.23$ (Figure 3). FSQ scores also significantly decreased over time, but without any significant group differences (Figure 4), main effect time $F(2,54) = 24.2$, $p < 0.001$; group x time $F(2,54) = 0.1$, $p = 0.90$. Similarly, speed in the BAT increased over time across groups, but hydrocortisone administration

did not yield any significant advantages compared to placebo (Figure 5), main effect time $F(2,54) = 33.7$, $p < 0.001$; group x time $F(2,54)=1.7$, $p = 0.19$.

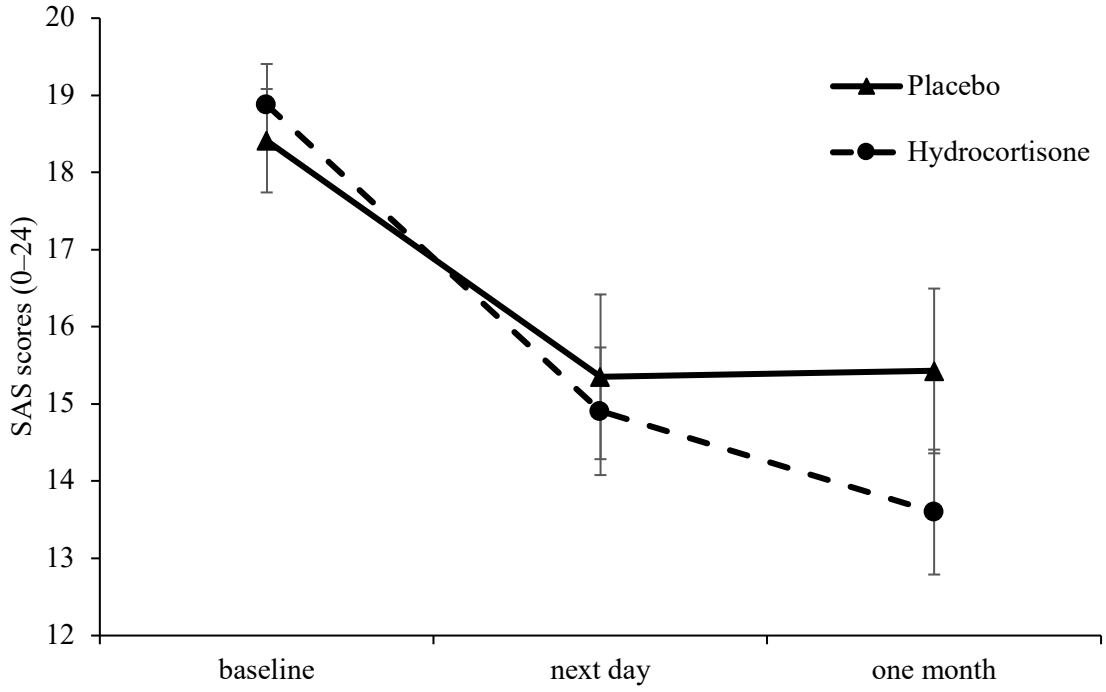

Figure 3. SAS scores at baseline, one-day and one-month follow-up in the two groups. SAS scores decreased significantly over time, but without significant group differences. Hydrocortisone administration was associated with a greater decrease in SAS scores compared to placebo during one-month follow-up, but this difference did not reach statistical significance. Error bars show standard error of the mean.

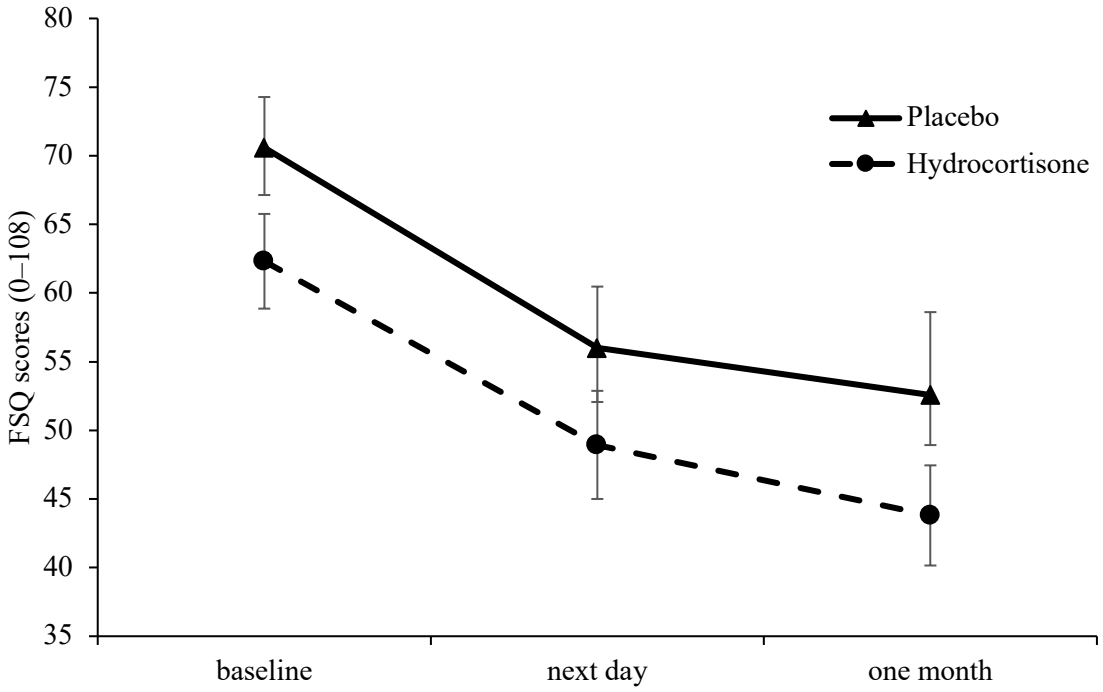

Figure 4. FSQ scores at baseline, one-day and one-month follow-up in the two groups. FSQ scores significantly decreased at one-day and one-month follow-up in both groups, but no significant group differences were obtained. Error bars show standard error of the mean.

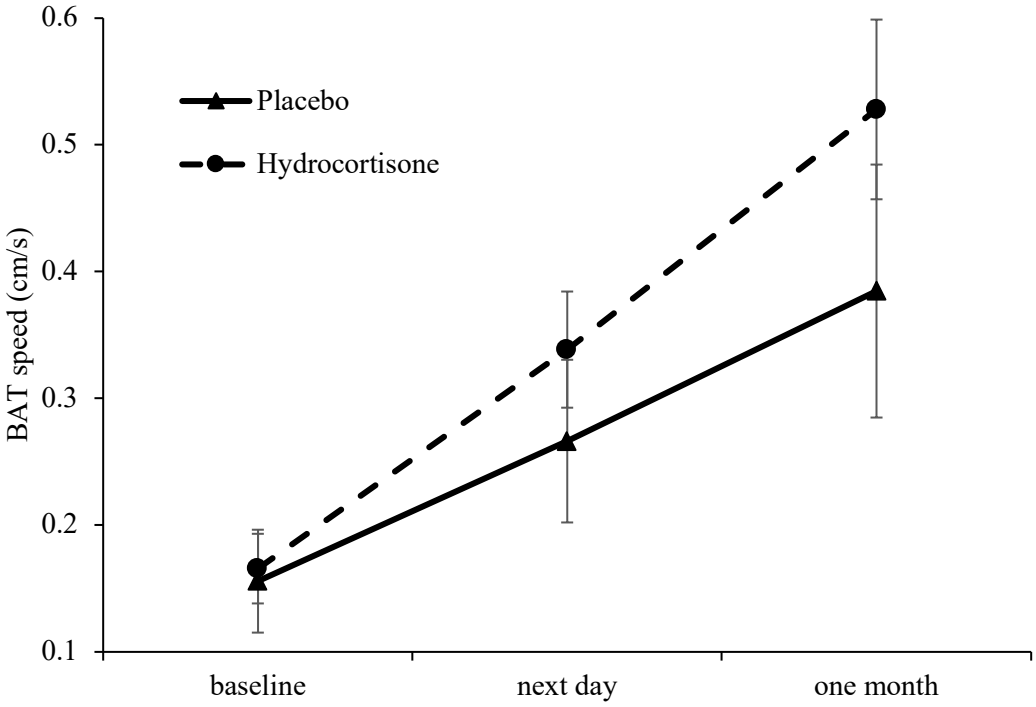

Figure 5. BAT speed at baseline, one-day and one-month follow-up in the two groups. BAT speed increased significantly over time but without any significant group differences. Although hydrocortisone administration did appear to be associated with greater increases in BAT speed compared to placebo, this group difference did not reach statistical significance. Error bars show standard error of the mean.

8.5 Cognitive bias measures – Extrinsic Affective Simon Task (EAST)

Only correct trials were included in the data analysis. As the butterfly trials were only included to present positive pictures in addition to the negative spider pictures, their analysis is not reported here. For each participant and each test time, median reaction time (RT) to spider images with the unpleasant key (compatible reaction) versus with the pleasant key (incompatible response) were calculated. EAST effects were computed by subtracting the RT with the pleasant key from the RT with the unpleasant key. A negative score indicates a negative evaluation; a positive score indicates a positive evaluation. These scores were entered into a time (baseline, next day, one-month follow-up) x group (hydrocortisone, placebo) mixed-model ANOVA.

Our results show that across groups the fear bias significantly reduced from baseline to one-day FU, main effect time $F(1,30) = 6.5$; $p = 0.016$. However, there was no significant difference in this reduction between the two groups, as the time x group interaction failed to reach the conventional level of significance $F(1,30) = 2.52$; $p = 0.123$ (Figure 6).

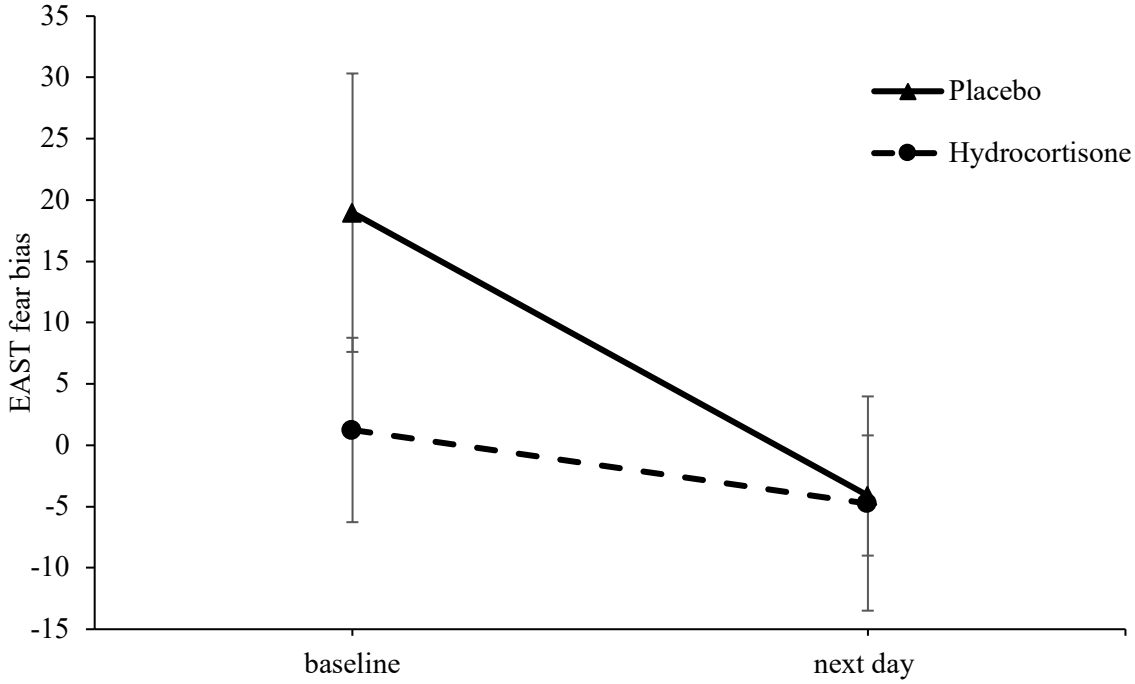

Figure 6. Mean EAST reaction time at baseline and one-day follow-up in the two groups. Fear bias significantly reduced on the day after treatment but there was no significant group difference on the day after CBT. Error bars show standard error of the mean.

8.6 Predictive value of cognitive bias for clinical symptom change

Mediation analyses (multiple linear regression) were run to establish whether changes in the fear bias on the day after treatment predicted symptom recovery during the one-month follow-up period. In the first step, one-day follow-up SAS scores were entered as a control variable of no interest. In the second step, one-day follow-up SAS scores as well as the predictors: group, one-day follow-up fear bias, and the group-fear bias interaction term were entered. The dependent variable was change on the SAS score between one-day follow-up and one-month follow-up. The same analysis was repeated for the FSQ and BAT.

Early changes in fear bias on the day after CBT treatment were not predictive of clinical change at one-month follow-up as measured using the SAS ($R^2 = 0.17$, $p = 0.24$), FSQ ($R^2 = 0.05$, $p = 0.99$) or BAT ($R^2 = 0.26$, $p = 0.77$).

9. Discussion

The aim of the current study was to investigate the effect of hydrocortisone administration compared to placebo on the clinical efficacy of one-session CBT in a cohort of spider-fearful individuals. This study also aimed to determine the impact of hydrocortisone on fear-information processing and whether early alterations in the fear bias on the day after treatment were predictive of clinical outcome one month later. Overall, participants receiving hydrocortisone did not significantly differ from those receiving placebo on clinical (SAS and FSQ) or behavioural (BAT) outcome measures at one-month follow-up. The additional benefits of hydrocortisone administration were also not evident for cognitive-bias (EAST) measures on the day after treatment. The results also did not support the notion that changes in fear bias on the day after treatment were predictive of clinical symptomatic improvement one month later in either the hydrocortisone or placebo group.

9.1 Lack of impact of hydrocortisone on clinical symptom outcomes

Although hydrocortisone administration did appear to yield a group difference in terms of the main clinical and behavioural outcome measures (Figure 3: SAS, Figure 4: FSQ,

Figure 5: BAT), this impact did not reach the conventional level of significance. There are various reasons why this study might not have demonstrated a hydrocortisone augmentation effect. Firstly, the effectiveness of the CBT intervention may have reached a ceiling, leading to smaller effect sizes, which could not be detected using the power in this study. This is consistent with previous literature, particularly in studies involving relatively simple treatment of specific phobias (Guastella et al. 2007; Nave et al. 2012; Tart et al. 2013). It is possible that increasing the sample size and therefore the power of the study would have enabled the clinical and behavioural differences seen in the hydrocortisone group compared to the placebo group to reach statistical significance. Furthermore, it is plausible that hydrocortisone-enhanced clinical symptomatic recovery is more likely to occur in patients with more complex anxiety disorders, or in participants with clinically relevant specific phobia symptoms. Given that 69.7% of spider-fearful individuals in this study did not fulfil the criteria for a diagnosis of specific phobia (DSM-V), it is conceivable that this may have contributed to these nil findings.

It is noteworthy however that scores on both clinical outcome measures (SAS and FSQ) and on the behavioural approach task (BAT) significantly improved from pre-treatment to one-day follow-up and remained significant one month later. However, as the study did not include a no-treatment control group, we cannot distinguish whether this score improvement represents a clinically meaningful effect or simply test-retest phenomenon. One indication that our results were not simply due to test-retest effects is the similarity of the results obtained in this study to those obtained by Muller et al. In their randomised-control trial, 36 spider-fearful individuals received either a single 27-minute session of CBE to nine fear-eliciting spider images, or were allocated to the control group in which they viewed nine neutral images. Compared to controls, individuals receiving CBE achieved significantly greater FSQ score reduction post-treatment (same day), and this group difference remained significant at one-month follow-up. Further, whilst the reduction in FSQ scores remained stable in the CBE group from post-treatment to one-month follow-up, control participants demonstrated a slight but non-significant increase in their FSQ scores (Figure 7, Müller et al. 2011). If we compare our results (particularly the difference in placebo and hydrocortisone-allocated participants' SAS score reductions, Figure 3), to those of Muller and colleagues we can observe a similar early treatment effect and reduction in fear over

time. Moreover, similar to Muller, our results also indicate a small, but non-significant increase in the control participants' SAS scores from one-day to one-month follow-up (Figure 3).

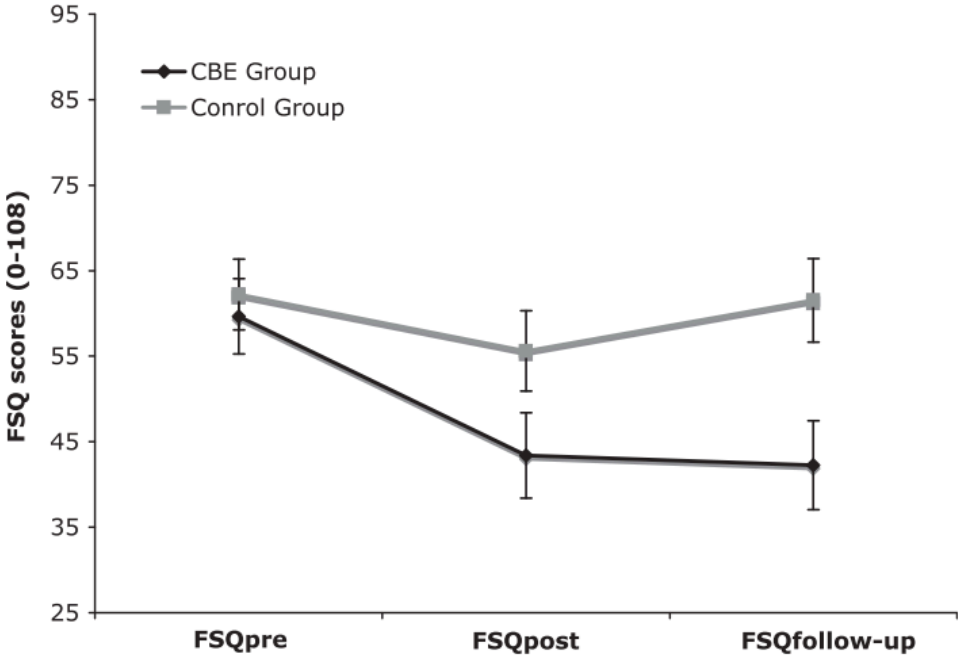

Figure 7. Mean FSQ scores at pre-treatment, post-treatment and one-month follow-up in the CBE and control group in Müller et al. 2011.

It is important to highlight that although in contrast to the first hypothesis presented in this study, the nil findings do not directly contradict previous literature. This is the first study to examine the effects of hydrocortisone administration on one-session CBT (comprising computer-based exposure and a therapist-guided component) in a cohort of spider-fearful individuals. The rationale behind this study was predominantly based on previous works revealing i) the success of one-session computer-based exposure treatment in spider-fearful individuals compared to controls (Müller et al. 2011) and ii) the benefits of administering glucocorticoids compared to placebo in combination with virtual reality exposure to heights in patients with specific fear of heights (De Quervain et al. 2011). Our motivation to conduct this study was that combining the success of CBE initiatives with the added benefits of exogenous glucocorticoid administration could lead to additional treatment gains. However, it is possible that the simple addition of treatment ingredients was not as feasible as we first imagined, and that exogenous hydrocortisone administration could not augment the results of the low-intensity CBT used in this study.

9.2 Combining computerised and therapist-guided exposure

A recent meta-analysis of computerised CBT (CCBT) programs for anxiety and depression found that studies with additional therapist support were more effective than those without (Spek et al. 2007). Further, a review of internet-based CBT programmes has identified a significant relationship between therapist support time and programme outcomes (Palmqvist et al. 2007), an effect which might be mediated by increased program engagement. Titov et al. conducted a randomised-control trial of CCBT for patients with social phobia, in which participants assigned to the clinician-assisted CCBT group displayed greater symptomatic improvement than self-guided CBT patients or those assigned to waiting list (Titov et al. 2008). In light of accumulating evidence underscoring the benefits of therapist guidance with CCBT initiatives, we hypothesised that the therapist-led CBT component in addition to the CBE element would present several advantages to our study. These included: i) developing an alliance with the therapist which may motivate the participant to continue exposure despite strong physiological, emotional and cognitive fear responses and ii) allowing the participant to better understand the anxiety-maintaining role of avoidance, enabling them to approach the CBE as a behavioural experiment, rather than having to rely on neurological pathways of fear extinction through exposure alone. However, by combining the computerised and therapist-led treatment ingredients we were unable to disentangle the benefits (if any) of each separate component. It is possible that by adding the therapist-led element we contributed to reaching a ceiling effect, leading to smaller effect sizes, such that the addition of hydrocortisone could not yield a significant enough effect to be detected using the power of this study. Perhaps had we only included the CBE component, we may have been able to observe the added benefits of hydrocortisone in comparison to the placebo-treated participants.

9.3 Side effects of hydrocortisone administration

With the exception of self-reported alertness ($p < 0.04$), heart rate ($p < 0.04$) and systolic blood pressure ($p < 0.03$), participants receiving hydrocortisone did not demonstrate any significant differences in side effects compared to the placebo group from baseline to drug peak level (Table 2). Although a greater decrease in heart rate (Figure 2) and systolic blood pressure was apparent in the hydrocortisone group compared to those receiving placebo, the group differences at baseline and drug peak level did not reach significance. Accordingly, participants in either group did not differ in how likely they were to report

that they had been given the drug (placebo group: 3/17, hydrocortisone group: 4/16, chi-squared $p = 0.46$), suggesting that differences in alertness, heart rate and systolic blood pressure between the groups were very subtle.

In line with these findings, there is scarce evidence for a link between corticosteroid administration and low blood pressure or bradycardia. A few isolated case reports have demonstrated that intravenous corticosteroid administration can trigger bradycardia or hypotension (Stroeder et al. 2015; Al Shibli et al. 2012; Soni et al. 2017), however, evidence for this phenomenon is on the whole scarce The mechanisms underpinning the potential link between intravenous corticosteroid injection and lowered heart rate and blood pressure are unknown but are thought to involve altered sensitivity of the sino-atrial node to catecholamines or altered cardio-myocyte contraction threshold due to electrolyte shift across the cell membrane (Hall et al. 1983; Fujimoto et al. 1990). It is important to note that the key difference between these case reports and our study is their use of intravenous corticosteroid administration, in comparison to this study, in which the glucocorticoid hydrocortisone was taken orally.

9.4 Strengths and limitations

Although this study has certain strengths, including the measurement of symptom change not only in terms of self-reported anxiety but also using in-vivo behavioural challenge tests, certain methodological limitations also warrant attention. Primarily, the study was conducted in a non-clinical population of spider-fearful individuals, with only 30.3% of the participant cohort fulfilling the criteria for a specific phobia (DSM-V). Although no differences in effect sizes have been reported in previous self-help studies using either self-report measures or a diagnostic interview to determine the presence of an anxiety disorder (e.g. Hirai & Clum 2006), it is possible that the overall intensity of the spider fear present in the participant cohort was too low for the effects of hydrocortisone to reach significance. Furthermore, our sample size was small, thus regression analyses are susceptible to false results and the generalisability of our findings is potentially limited. A limitation of the computer-based exposure (CBE) therapy element is the possibility that that not all of the subjects fully engaged in the self-directed task and may have adopted defensive mechanisms such as escape and avoidance, to alleviate stress (McNaughton & Corr 2004). Although, our study aimed to minimise the likelihood of this by instructing participants to click on a series of stars on various parts of the spiders'

bodies and telling the subjects that their responses were being monitored, we cannot exclude the possibility that avoidance behaviours occurred during the CBE.

9.5 Future directions

The present study would benefit from various refinements and improvements. Notably, a larger participant cohort would have been advantageous so that all clinical and cognitive bias outcome measures could have been better powered after the removal of outliers. In addition, longer follow-up periods for example after six months and one year, would have potentially enabled additional or longer-term conclusions to be drawn about the mechanisms and trajectories of change following treatment. As previously mentioned, it would be desirable to conduct the study in a clinically diagnosed phobic population or in patients with more complex anxiety disorders, and doing so may offer explanation for the nil findings of this study. Finally, using eye-tracking measurements to delineate whether attentional shifts during the CBE are due to orienting or disengagement mechanisms (Armstrong, Thomas 2012) would enable us to test the hypothesis that potentially not all participants fully engaged in the CBE.

10. Conclusions

Overall, the administration of hydrocortisone was not found to improve clinical, behavioural or cognitive bias outcome measures compared to placebo following one-session of CBT in a cohort of spider-fearful individuals. No predictive value for early changes in fear-information processing on the day after treatment were revealed for clinical symptom change one month later. Further research is required to elucidate specifically which factors have underpinned the hydrocortisone augmentation effect seen in other studies using exposure-based CBT for anxiety disorders in the past (e.g. Quervain et al. 2011). An improved understanding of the mechanisms underlying effective treatment outcome will better inform the design of future studies and will ultimately guide the development of enhanced therapeutic tools for anxiety disorders.

Chapter 3 - The interaction between endogenous cortisol and cortisone, fear extinction and fear information processing

11. Introduction

11.1 Endogenous glucocorticoids and anxiety disorders

The hypothalamic-pituitary-adrenal (HPA) axis and its end product cortisol are key players in mediating the body's response to stress (Cacioppo et al. 2016). Whilst alterations to the HPA axis are a common pathophysiological finding in individuals with anxiety, there appears to be no unifying disturbance across the spectrum of anxiety disorders (Elnazer & Baldwin 2014).

A major limitation of current CBT initiatives is their inefficacy in a subgroup of patients. One way to combat this would be to identify mechanistic markers that dictate whether a patient is likely to respond to treatment or not. One candidate marker is the endogenous glucocorticoid hormone cortisol. Previous work has revealed that higher cortisol levels during exposure predict improved clinical efficacy of psychotherapeutic interventions in patients with various anxiety disorders (Siegmund et al. 2011; Meuret et al. 2015; Gaab et al. 2005). Additionally, it has been shown that exposure therapy in spider phobic individuals is more effective in the morning than in the evening, likely to due to higher endogenous cortisol levels (Lass-Hennemann & Michael 2014). In the context of PTSD, evidence also suggests that elevated pre-treatment cortisol and enhanced pre-treatment HPA negative feedback predict greater post-treatment symptomatic improvements (Yehuda 2009; Yehuda et al. 2014; Nijdam et al. 2015).

Research also indicates that cortisol elevations prior to therapy can predict improved clinical results (Yehuda 2009; Yehuda et al. 2014; Colvonen et al. 2017). The cortisol-awakening response (CAR) represents a distinct aspect of diurnal HPA axis functioning and can be defined as the period of cortisol secretory activity in the first 45 to 60 minutes immediately post-awakening (Clow et al. 2004). However, whilst one study has revealed that a greater CAR is associated with enhanced clinical outcomes two months after treatment (Meuret et al. 2015), other studies have not been able to demonstrate an association between CAR and treatment efficacy (Brand et al. 2011; Dierckx et al. 2012).

11.2 The interaction between endogenous and exogenous glucocorticoids in anxiety disorders

As mentioned in the previous chapter, an elegant series of studies in patients with different kinds of phobia has revealed that exogenous glucocorticoid administration can enhance the efficacy of exposure-based therapies (Soravia et al. 2006; De Quervain et al. 2011; Soravia et al. 2014). However, only more recently have we begun to appreciate the interaction between glucocorticoid-enhanced outcomes and endogenous glucocorticoid functioning. For example, it has been observed that PTSD patients who benefitted the most from glucocorticoid-enhanced exposure therapy were those with highest pre-treatment glucocorticoid sensitivity (Yehuda et al. 2015).

11.3 Endogenous glucocorticoids and attentional bias

Considering that normalising the fear bias appears to be prerequisite for clinical symptomatic changes during CBT for anxiety disorders (Reinecke et al. 2012; Reinecke, Waldenmaier, et al. 2013; Reinecke, Rinck, et al. 2013), the possibility arises that endogenous glucocorticoid functioning may also interact with the fear bias interface during treatment. Previous work has demonstrated that higher endogenous baseline cortisol levels are associated with lower attentional bias towards angry faces in healthy adults (Van Honk et al. 1998; Van Honk et al. 2000). However research linking endogenous HPA function to the fear bias interface in individuals with anxiety disorders is scarce.

12. Objectives and hypotheses

This study aimed to measure cortisol and cortisone concentrations from hair and saliva samples to provide indications of long-term and acute glucocorticoid levels, respectively. Samples were obtained before, during and after exposure-based CBT in cohort of participants with higher spider fear who were randomly allocated to receive either hydrocortisone or placebo.

The following hypotheses were tested:

(i) Hydrocortisone administration will result in an acute increase in salivary cortisol and cortisone.

Compared to placebo, hydrocortisone administration led to significantly higher salivary cortisol levels between 60 and 150 minutes after capsule intake (Soravia et al. 2014).

(ii) Greater salivary cortisol and cortisone levels during treatment predict enhanced clinical efficacy of one-session CBT at one-month follow-up.

Greater absolute cortisol levels during psychotherapeutic treatment for anxiety disorders correlate with enhanced clinical outcome (Siegmund et al. 2011; Meuret et al. 2015; Gaab et al. 2005; Lass-Hennemann & Michael 2014).

(iii) Greater morning rises in salivary cortisol and cortisone levels predict the clinical efficacy of one-session CBT at one-month follow-up.

Rises in cortisol levels on the morning of psychotherapeutic interventional therapy are predictive of therapeutic outcome in patients with panic disorder and agoraphobia (Meuret et al. 2015).

(iv) Baseline endogenous hair cortisol and cortisone concentrations predict the clinical efficacy of one-session CBT at one-day and one-month follow-up.

Hair analysis provides a reliable, retrospective indicator of cumulative, long-term cortisol and cortisone secretion over a period of several months (Russell et al. 2012; Stalder et al. 2012).

(v) Baseline endogenous hair cortisol and cortisone concentrations predict the magnitude of change in fear bias at one-day follow-up.

Higher endogenous baseline cortisol levels are associated with lower attentional bias towards angry faces in healthy subjects (Van Honk et al. 1998; Van Honk et al. 2000).

Overall, this study will provide an insight into the interaction between the endogenous glucocorticoid system, exogenous hydrocortisone administration and clinical symptomatic improvement following exposure-based CBT. This may reveal the previously unrecognised value of using salivary or hair cortisol concentration analysis as a novel tool for the early prediction of exposure-based treatment efficacy in patients with anxiety disorders.

13. Methods

In addition to the methods detailed in chapter 1, further techniques used in this study are defined below.

13.1 Cortisol and cortisone analysis

For several years, determination of cortisol concentration in saliva has been a widely accepted and commonly employed technique in psychoneuroendocrinological research, and previous work has validated and evaluated methods of measuring salivary cortisol (e.g., Kirschbaum & Hellhammer, 1989, 1994). Compared to blood analyses, salivary cortisol measurements boast several advantages including: stress-free samping, laboratory independence and lower costs. Whilst salivary cortisol concentration does provides a reflection of acute circulating cortisol levels in the blood, it fails to inform about cumulative cortisol secretion over longer periods. The analysis of hair cortisol concentration is a recent methodological development aimed at providing some advancement in this domain. Given that lipophilic substances are continuosly incorporated into growing hair, hair cortisol measurements are assumed to provide an easily obainable index of cumulative cortisol secretion over an extended period of several months (Russell et al. 2012; Stalder et al. 2012). The rationale behind this idea has been supported by studies revealing that hair cortisol concentration analysis has high test-retest reliability (Shorta et al. 2016; Stalder et al. 2012) and acute/situational stability (Grass et al. 2015). Hair sampling is also non-invasive, easily performed and generally well-tolerated (Stalder et al. 2012). This study will also examine salivary and hair cortisone concentration, given evidence to suggest that salivary cortisone may provide a more detailed and robust reflection of systemic cortisol concentration than salivary cortisol measurements (Perogamvros et al. 2010).

13.2 Saliva sampling and analysis

On the morning of the first testing visit, participants collected three saliva samples at home using salivettes (Sarstedt Inc. Rommelsdorf. FRG). The first sample was taken immediately after waking up, the second after 30 minutes and the final sample was taken 45 minutes after waking. Participants were instructed to take nothing by mouth other than water, to refrain from smoking or brushing their teeth, and to otherwise follow their normal routine until all three saliva samples were taken. Salivettes comprised a sterilised

cotton swab, contained within a small beaker, which together fitted within a simple plastic tube. Saliva samples were obtained by chewing gently on the cotton swab for 30 to 60 seconds, which was and subsequently placed in the beaker, and secured in the plastic tube. Participants were asked to record the exact time that each sample was taken, to store the three salivettes in the fridge and bring them to the testing visit. In order to increase the likelihood that participants adhered to the requested protocol and took their saliva samples at the correct times, subjects were given the three salivettes in a mock MEMS 6 TrackCap container (Aardex Ltd., Zug, Switzerland). The participants were under the impression that the mock containers were real and were able to track the times at which the containers were opened and the saliva samples were obtained.

Throughout the first testing visit, seven additional saliva samples were obtained. These were taken at the baseline, immediately after the BAT, 15 minutes after the BAT, at drug peak level, after computer-based exposure (CBE), after therapist-guided exposure and at the end of the session. During the one-month follow-up visit, 3 additional samples were taken. These were obtained at baseline (start of session), immediately after the BAT and at the end of the session (15 minutes after the BAT). All anonymised saliva samples were stored at -20°C in the laboratory freezer until biochemical analysis. After thawing, saliva samples were centrifuged for 10 minutes at 4000 rpm. 100 µL saliva was added to a tube together with 50 µL cortisol-D_4 (internal standard) and 150 µL methanol/water containing 50 mg/mL zinc sulfate (vol/vol: 50/50). After vortexing for 1 minute and centrifugation for 5 minutes at 12000 rpm, 200 uL supernatant was injected into the LC-MS/MS system. The LC-MS/MS system consisted of a Shimadzu LC-20AD high-pressure liquid chromatography unit, a Shimadzu SIL-20AC autosampler, and a Shimadzu CTO-20AC column temperature oven (Shimadzu Europa GmbH, Duisburg, Germany), which was coupled to an AB Sciex API 5000 turbo ion spray triple quadrupole tandem mass spectrometer (AB Sciex Germany GmbH, Darmstadt, Germany). The system was controlled by AB Sciex Analyst software (version 1.5.1). Intra-assay and inter-assay coefficients of variance have been calculated to be between 3.7% and 8.8% (Gao et al. 2013)

13.3 Hair sampling and analysis

At the start of the first testing visit, hair strands (~3 mm diameter) were cut as close as possible to the scalp from a posterior vertex position. The scalp-near 1 cm hair segment

was analysed. Based on an average hair growth rate of 1 cm per month (Wennig 2000), hair cortisol concentration in this segment is thought to reflect cumulative cortisol secretion over the previous one-month period.

Washing and steroid extraction procedures followed the protocol described by Gao et al. (Gao et al. 2013). 10 mg of whole, non-pulverised hair was washed by shaking the sample in 2.5 mL isopropanol for 3 minutes at room temperature. The sample was then allowed to dry completely under a fume hood for at least 12 hours. 10 mg of washed, dry hair was transferred into a 2 mL tube (Eppendorf, Hamburg, Germany) for steroid extraction. The hair sample was incubated for 18 hours at 45°C with 50 µL of cortisol-D_4 (internal standard) and 1800 µL methanol before being spun in a centrifuge at 10,000 rpm for 2 minutes 1000 µL of the clear supernatant was transferred to a new 2 mL tube and the alcohol was evaporated at 65°C under a constant stream of nitrogen until the samples were completely dried (approximately 20 minutes). The dry residue was resuspended using 250 µL double-distilled water, 200 µL of which was used for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis.

13.4 Liquid chromatography tandem mass spectrometry

As mentioned above, hair and salivary glucocorticoid analysis was carried out using LC-MS/MS. In this method, the sample mixture is first separated by liquid chromatography, before being ionised and characterised by mass-to-charge-ratio and relative abundance by two mass spectrometers in sequence. This method is extremely sensitive, enabling detection of very low concentrations of analyte. It is also highly specific thus can distinguish between structurally similar compounds (Grebe & Singh 2011).

13.5 Statistical analysis

Statistical analyses were performed using SPSS for Windows, version 22 (IBM, Chicago, Illinois) at the Department of Psychiatry, University of Oxford. In order to test the hypothesis that hydrocortisone administration results in an acute increase in salivary cortisol and salivary cortisone, mixed-model ANOVAs with the within-subjects factor time and the between-subjects factor group were used. To further explore the nature of any interactions arising in this step, one-sided, independent samples t-tests were used to determine at which point(s) during the testing session cortisol and cortisone levels differed significantly between the two groups. To test the hypothesis that salivary cortisol

and cortisone levels during treatment correlate with improved clinical efficacy of one-session CBT at one-month follow-up, mediation analysis (multiple linear regression) were performed as per Kraemer et al. controlling for initial baseline clinical severity (Kraemer et al. 2002). Mediation analyses were also performed to determine whether morning rises in cortisol and cortisone concentrations were predictive of improved clinical outcome in the month following treatment, controlling for initial baseline clinical severity. Separate stepwise regression analyses were conducted to evaluate the predictive value of baseline hair cortisol and cortisone for clinical symptom improvement at one-day and one-month follow-up. Regression analyses were also performed to determine the predictive value of baseline hair cortisol and cortisone for the magnitude of change in fear bias the day after treatment. To further explore the nature of any interaction arising between baseline hair cortisol/cortisone and clinical improvement or the magnitude of change in fear bias, follow-up simple regression analyses were run separately for the two groups.

14. Results

14.1 Effect of hydrocortisone administration on salivary cortisol and cortisone

Mixed model ANOVAs with the within-subjects factor time (baseline, after BAT, 15 minutes after BAT, 1 hour after drug/placebo, after CBE, after therapist-guided exposure, end of session) and the between-subjects factor group were used to determine if hydrocortisone administration resulted in an acute increase in salivary cortisol and cortisone. Compared to the placebo group, hydrocortisone administration resulted in an acute increase in salivary cortisol: significant time x group interaction $F(6,126) = 16.0$, $p < 0.001$ (Figure 8) and cortisone: $F(6,126)$, $p < 0.001$ (Figure 9). To further explore the nature of this interaction, one-sided independent samples t-tests were run to determine at which timepoint(s) cortisol and cortisone levels differed significantly between the two groups. Compared to baseline, hydrocortisone administration resulted in an acute increase in salivary cortisol and cortisone in the drug group compared to the placebo group one hour after capsule intake. Cortisol: $t(23) = -2.0$; $p = 0.03$ and cortisone: $t(23) = -2.0$; $p = 0.03$. As shown in Figures 8 and 9, cortisol and cortisone concentrations continued to rise after this one-hour mark, however we cannot delineate

whether this was due to prolonged absorption of hydrocortisone, or due to endogenous glucocorticoid release following the CBE initiative.

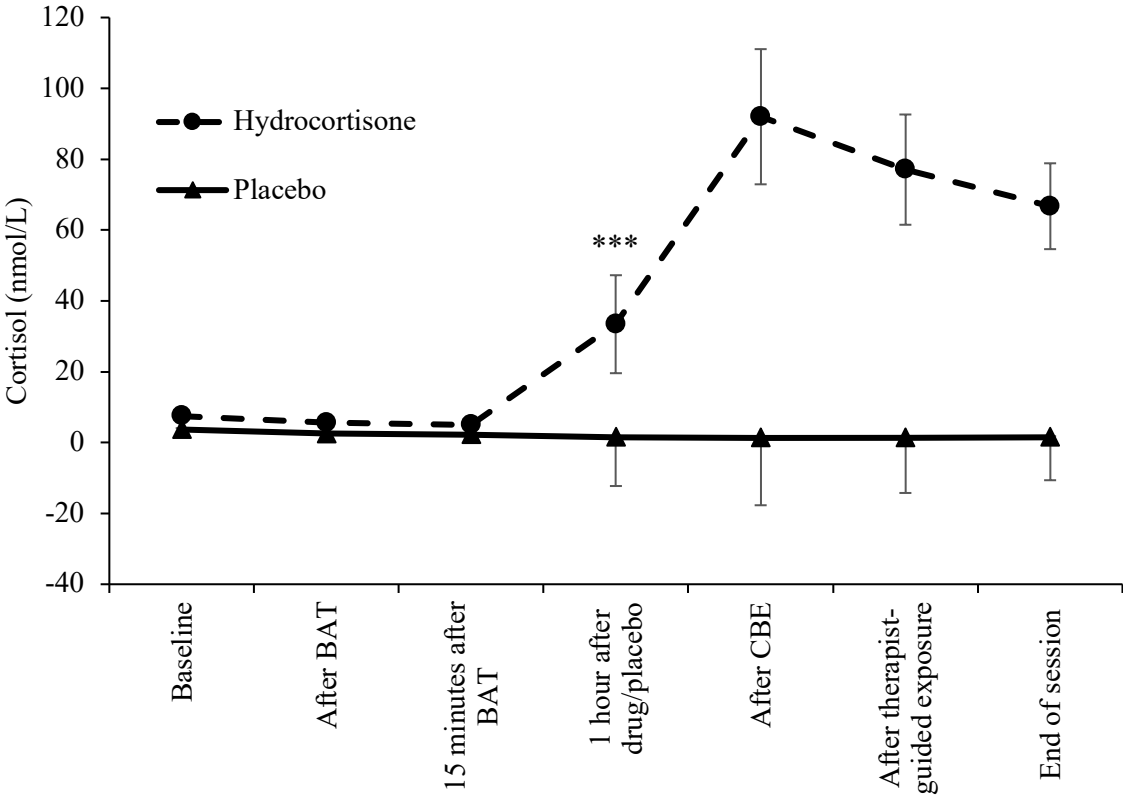

Figure 8. **Salivary cortisol levels measured at seven timepoints during the testing session.** The administration of hydrocortisone led to significantly higher cortisol levels in the drug group compared to the placebo group ($p < 0.001$). Error bars show standard error of the mean. Asterisks indicate significant differences one hour after capsule intake compared to baseline ($p < 0.001$). CBE; computer-based exposure.

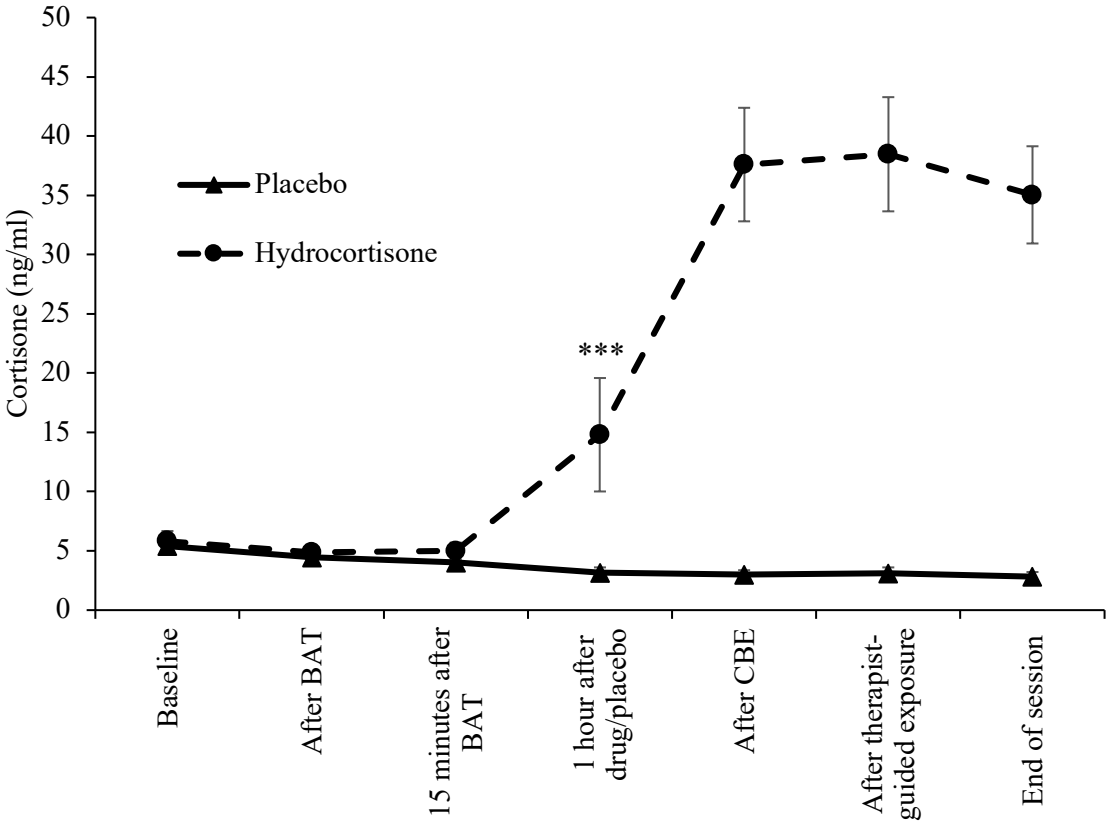

Figure 9. **Salivary cortisone concentrations obtained at seven timepoints throughout the testing session**. Compared to the placebo group, hydrocortisone administration resulted in a significant increase in salivary cortisone ($p < 0.001$). Asterisks indicate significant differences one hour after capsule intake compared to baseline ($p < 0.001$). CBE; computer-based exposure. Error bars indicate standard error of the mean.

14.2 Predictive value of salivary cortisol and cortisol levels during treatment for clinical recovery

Previous evidence suggests that higher endogenous cortisol levels (not drug-induced) during *in-vivo*, exposure-based therapy correlate with enhanced clinical outcome. This has been demonstrated in in patients with specific phobia (Lass-Hennemann & Michael 2014; Gaab et al. 2005) and subjects with panic disorder and agoraphobia (Siegmund et al. 2011; Meuret et al. 2015). Given these findings, one aim of this study was to establish whether salivary cortisol and cortisone levels during the brief CBT treatment could predict symptom recovery during the one-month follow-up period. Two mediation analyses (multiple linear regression analyses) were run, one for cortisol and one for cortisone measures. In each of these analyses, baseline SAS scores were entered in the first step as a control variable of no interest. Baseline SAS scores and the predictors: group, the group-cortisol interaction term and cortisol during treatment were

entered in the second step. The dependent variable was change on the SAS score between baseline and one-month follow-up. The same analysis was repeated for the FSQ and BAT and also using salivary cortisone as the predictor. Salivary cortisol and cortisone levels "during exposure" were calculated as the average of four samples: one hour after drug/placebo (which was just before psychoeducation), after CBE, after therapist-guided exposure and end of session, as per previous work (Siegmund et al. 2011; Meuret et al. 2015).

Salivary cortisol levels during the CBT session did not predict improved scores on the SAS ($R^2 = 0.21$; $p = 0.99$), FSQ ($R^2 = 0.12$; $p = 0.97$) or BAT ($R^2 = 0.40$; $p = 0.47$). Improved clinical response was also not predicted by salivary cortisone concentration during treatment (SAS: $R^2 = 0.23$; $p = 0.67$, FSQ: $R^2 = 0.11$; $p = 0.84$ and BAT: $R^2 = 0.38$; $p = 0.82$).

14.3 Predictive value of morning rises in salivary cortisol and cortisone for clinical recovery

Previous research indicates that rises in cortisol levels on the morning of psychotherapeutic interventional therapy are predictive of therapeutic outcome (Meuret et al. 2015). In this study, mediation analyses were performed to determine whether the salivary cortisol and cortisone awakening response could predict symptom recovery during the month following treatment. Two analyses were run, one for cortisol and one for cortisone salivary measurements. In each of these analyses, baseline SAS scores were entered in the first step as a control variable of no interest. Baseline SAS scores and the predictors: group, the group-morning cortisol rise interaction term and morning cortisol rise were entered in the second step. The dependent variable was change on the SAS score between baseline and one-month follow-up. The same analysis was repeated for the FSQ and BAT and also using salivary cortisone as the predictor. Given that the cortisol awakening response can be defined as the period of cortisol secretory activity in the first 45 to 60 minutes immediately post-awakening (Clow et al. 2004), we calculated morning salivary cortisol and cortisone rises by subtracting concentrations immediately after waking from those obtained 45 minutes after waking.

Morning cortisol rises did not predict improved scores on the SAS ($R^2 = 0.34$; $p = 0.56$), FSQ ($R^2 = 0.06$; $p = 0.94$) or BAT ($R^2 = 0.41$; $p = 0.28$). Improved clinical

outcome was also not predicted by salivary cortisone rises on the morning of treatment (SAS: $R^2 = 0.18$; $p = 0.82$, FSQ: $R^2 = 0.04$; $p = 0.66$ and BAT: $R^2 = 0.47$; $p = 0.76$).

14.4 Predictive value of baseline hair cortisol and cortisone for the clinical efficacy of one-session CBT

Mediation analyses were run to determine whether baseline (pre-treatment) endogenous hair cortisol and cortisone concentrations could predict clinical improvements following one-session CBT. Two analyses were run, one for cortisol and one for cortisone. In each of these analyses, baseline SAS scores were entered in the first step as a control variable of no interest. Baseline SAS scores and the predictors: group, the group-hair cortisol interaction term and baseline hair cortisol were entered in the second step. The dependent variable was change on the SAS score between baseline and one-month follow-up. The same analysis was repeated for the FSQ and BAT and also using baseline endogenous hair cortisone as the predictor.

Baseline endogenous hair cortisol levels were not found to predict clinical symptomatic recovery at one-month follow-up: (SAS: $R^2 = 0.22$; $p = 0.70$, FSQ: $R^2 = 0.14$; $p = 0.61$ and BAT: $R^2 = 0.45$; $p = 0.13$). Similar findings were obtained for endogenous hair cortisone concentration which also did not predict the clinical efficacy of one-session CBT after one-month (SAS: $R^2 = 0.22$; $p = 0.66$, FSQ: $R^2 = 0.19$, $p = 0.28$ and BAT:$R^2 = 0.41$; $p = 0.85$). However, the analysis did suggest a trend for baseline hair cortisone to predict FSQ score reductions differently in either the placebo or hydrocortisone group (significant group x cortisone interaction, $p = 0.057$). To further explore this non-significant interaction, follow-up simple regression analyses were run separately for the two groups. Results indicated a non-significant trend in the drug group ($p = 0.083$) but not the placebo group ($p = 0.56$). This suggests that lower baseline hair cortisone predicted enhanced clinical outcome in individuals who received hydrocortisone, or possibly that participants in the hydrocortisone group with lower baseline hair cortisone benefited more from exogenous hydrocortisone administration than those with higher baseline hair cortisone (Figure 10).

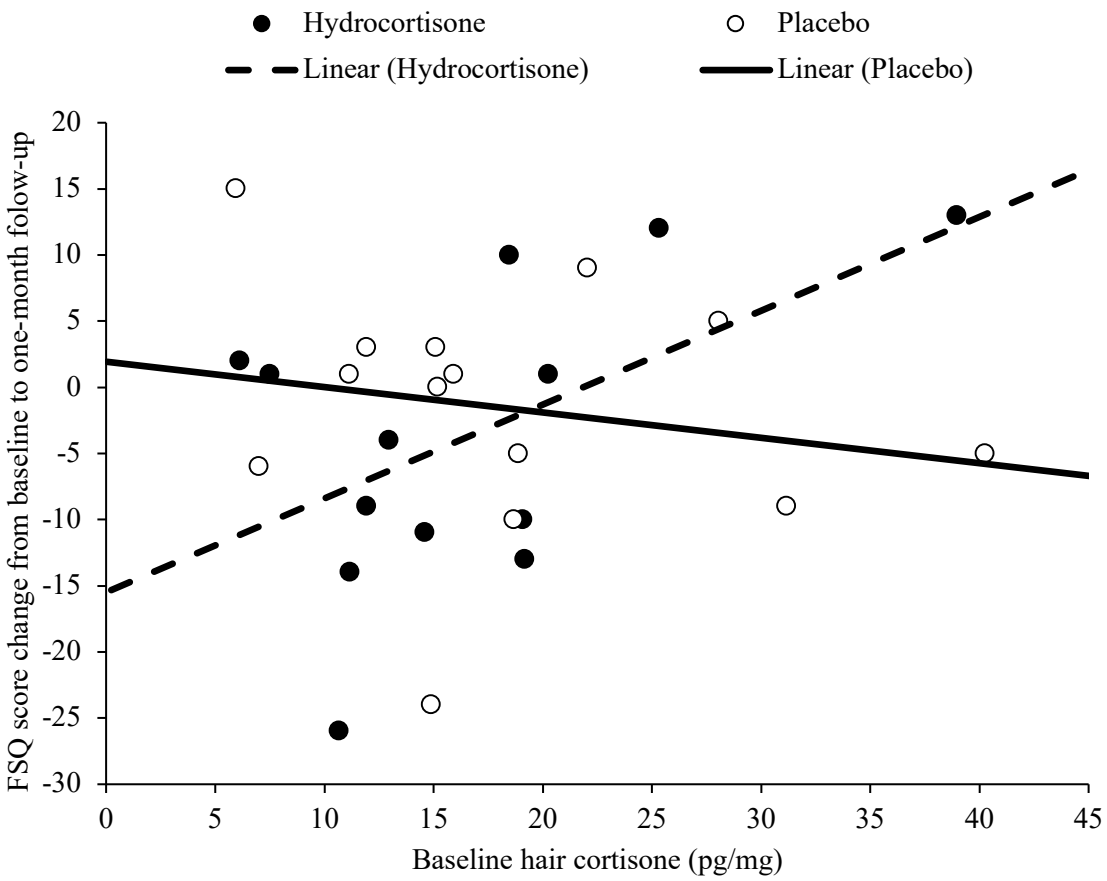

Figure 10. Predictive value of baseline hair cortisone for FSQ score change at one-month follow-up. In participants receiving hydrocortisone, there was a non-significant trend for lower baseline hair cortisone to predict improved FSQ scores.

14.5 Predictive value of baseline hair cortisol and cortisone for the magnitude of change in fear bias

Mediation analyses (multiple linear regression) were run to establish whether baseline hair cortisol predicted change in fear bias from baseline to one-day follow-up. Baseline EAST scores were entered in a first step as a control variable of no interest, and the predictors: group, baseline hair cortisol, and the group-hair cortisol interaction term were entered in a second step. The dependent variable was change in fear bias, as measured by changes in EAST reaction times (RT) from baseline to one-day follow-up. The same analysis was repeated for cortisone as the predictor.

Baseline hair cortisol did not predict change in the fear bias at one-day follow-up in either the hydrocortisone or placebo group ($R^2 = 0.62$; $p = 0.37$). Baseline hair cortisone significantly predicted change in fear bias across the groups ($R^2 = 0.68$; $p = 0.037$), such that lower baseline hair cortisone predicted a greater change in fear bias. There

appeared to be a trend for this relationship to be stronger in one of the groups (significant group x cortisone interaction, $p = 0.09$) and to further explore this non-significant trend, follow-up simple regression analyses were run separately for each group. Results showed that lower baseline cortisone significantly predicted stronger change in the fear bias in the 24-hour period after treatment in the drug group ($p = 0.032$) but not in the placebo group ($p = 0.86$, Figure 11).

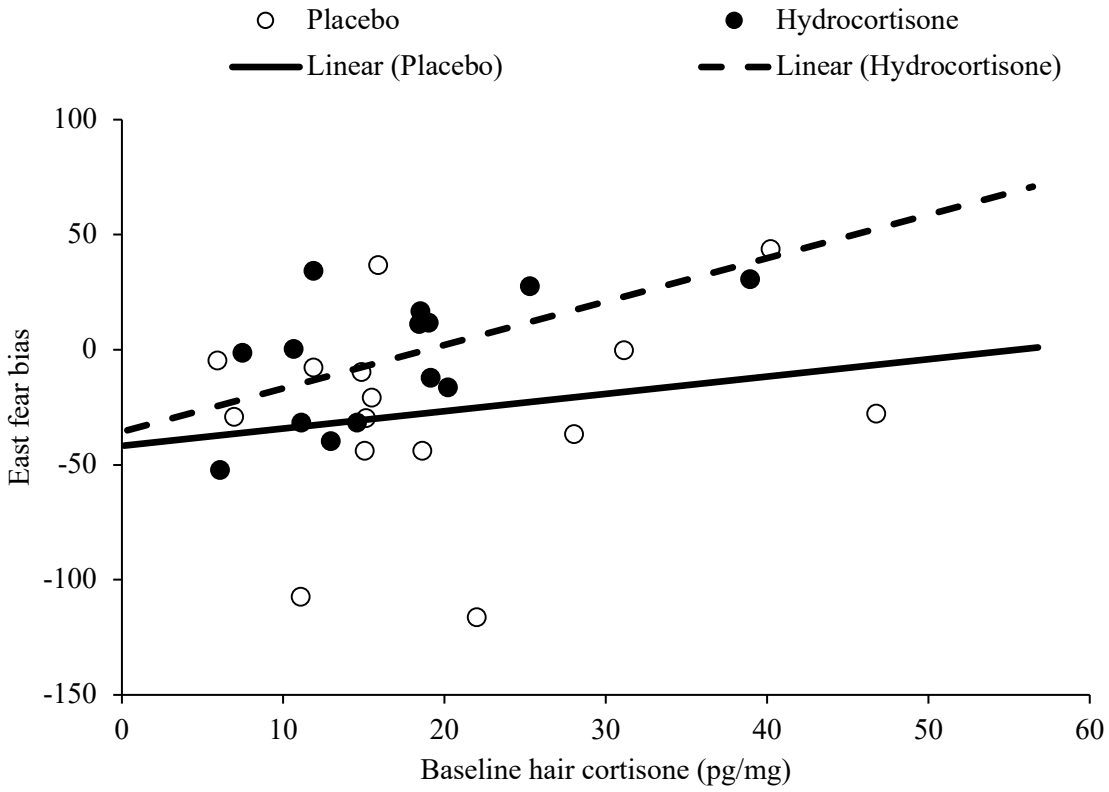

Figure 11. Predictive value of baseline hair cortisone for the magnitude of change in fear bias (EAST reaction time scores) at one-day follow-up. There was a non-significant trend for lower baseline hair cortisone to predict greater change in the fear bias in participants receiving hydrocortisone, compared to those receiving placebo.

15. Discussion

This study had several aims. One was to demonstrate that hydrocortisone administration resulted in an acute increase in salivary cortisol and cortisone concentrations in a cohort of spider fearful individuals. We also aimed to show that endogenous baseline salivary cortisol and cortisone concentrations on the morning of treatment and during treatment were predictive of clinical recovery during the month after treatment. This study also aimed to evaluate the predictive value of baseline hair cortisol and cortisone for i) the clinical efficacy of CBT at one-month follow-up and ii) the magnitude of change in fear bias on the day following treatment. Overall, the results indicate that compared to placebo, hydrocortisone administration caused an acute increase in salivary cortisol and cortisone concentrations as hypothesised. Salivary cortisol and cortisone measurements on the morning of treatment and during treatment were not predictive of the clinical efficacy of CBT at one-month follow-up. Endogenous baseline hair cortisol did not predict clinical symptomatic improvement after one-month. However, there was a non-significant trend for lower hair cortisone to predict enhanced clinical outcome at one month in the drug group, but not the placebo group. Baseline hair cortisol was not predictive of changes in the magnitude of fear bias on the day following treatment. Baseline hair cortisone significantly predicted change in fear bias across the groups and there was a non-significant trend for this relationship to be stronger in one of the groups. Indeed, lower baseline hair cortisone concentration significantly predicted greater change in the fear bias in the 24 hour period after treatment in the hydrocortisone group, but not the placebo group.

15.1 Hydrocortisone administration results in acute salivary cortisol and cortisone release

In line with our first hypothesis, hydrocortisone administration resulted in an acute increase in salivary cortisol and cortisone concentrations in the drug group compared to the placebo group one hour after capsule intake (Figures 8 and 9). After one hour, CBT commenced, thus after this point, our results cannot distinguish whether the rising salivary cortisol and cortisone concentrations observable in the hydrocortisone group were due to prolonged hydrocortisone absorption, or were in fact due to endogenous release triggered by the CBT treatment.

An alternative explanation for the finding that hydrocortisone resulted in an acute increase in salivary cortisol and cortisone is that hydrocortisone administration enhanced the sensitivity of these participants' endogenous HPA axes to the effects of CBT. This potentially meant that endogenous glucocorticoid release was more likely in these subjects compared to those receiving placebo. This theory is in line with previous findings demonstrating an interaction between the effects of exogenous and endogenous glucocorticoids during treatment of patients with PTSD (Yehuda et al. 2015).

15.2 Predictive value of salivary cortisol and cortisone release during treatment for clinical recovery

Previous work has revealed a correlation between higher absolute cortisol levels during *in-vivo* exposure-based CBT and more effective treatment outcome. This has been demonstrated in in patients with specific phobia (Lass-Hennemann & Michael 2014; Gaab et al. 2005) and subjects with panic disorder and agoraphobia (Siegmund et al. 2011; Meuret et al. 2015). Our results did not reveal a predictive value for salivary cortisone or cortisol release during treatment for the clinical efficacy of CBT at one-month follow-up. One explanation for these nil findings is that CBT did not take place at peak drug level (Figures 8 and 9). Based on previous literature in which 20mg hydrocortisone has been delivered one hour prior to exposure-based CBT interventions in patients with spider phobia (Soravia et al. 2014) and patients with specific fear of heights (De Quervain et al. 2011), we hypothesised that drug levels in this study would peak approximately one hour after capsule administration. Therefore, the CBT was delivered one hour after the participants took either the hydrocortisone or placebo. However, our results demonstrate that salivary cortisol continued to rise until after the computer-based exposure and salivary cortisone levels continued to increase until the therapist-guided exposure had terminated. The theory that the CBT initiative triggered endogenous cortisol release and thus was responsible for the observed rise in salivary cortisol and cortisone concentrations is unlikely for several reasons: Firstly, previous research has demonstrated a lack of cortisol release during exposure-based treatments for anxiety disorders. This includes studies in patients with specific phobia (Lass-Hennemann & Michael 2014) and those with panic disorder and agoraphobia (Siegmund et al. 2011; Meuret et al. 2015; Woods et al. 1987; Cameron et al. 1987). Moreover in this study, there were no significant changes in salivary cortisol or cortisone levels in the

placebo groups during the CBT compared to baseline. Taken together, it is more likely that the increase in salivary glucocorticoid concentration seen after the CBT intervention was due to slower than expected absorption of hydrocortisone, rather than the stress-related endogenous glucocorticoid-release during treatment. Perhaps had the CBT been delivered slightly later, at the actual drug peak level, we may have observed a significant difference between the hydrocortisone and placebo groups regarding their performance in clinical outcome measures at one-day and one-month follow-up (see Chapter 2). It is possible that had the CBT been delivered later, the results may also have revealed a predictive value for salivary cortisol and cortisone levels during treatment for symptomatic improvement during the month following treatment.

An alternative possibility for the finding that salivary cortisol and cortisone did not predict clinical outcome is that some of the participants in this study may have had dysfunctional HPA axes, given previous evidence to suggest that anxiety disorders are characterised by heterogenous HPA axis dysfunctions (Elnazer & Baldwin 2014). However, since only 30.3% of the total participant cohort fulfilled the DSM-V criteria for a specific phobia, it is unlikely that the severity of HPA axes malfunctions (if any) contributed to these nil findings.

Overall, it is important to note these findings showing that salivary cortisol or cortisone levels during treatment are not predictive of improved treatment outcome, are not in contrast with the previous literature. Although some previous studies have provided evidence for higher cortisol levels during exposure being linked to improved therapeutic response (Meuret et al. 2015; Lass-Hennemann & Michael 2014; Siegmund et al. 2011) other studies have not been able to confirm this (Brand et al. 2011). Indeed, an overall meta-analysis of studies evaluating cortisol as a predictor of psychological therapy response in anxiety disorders has shown no significant relationship between stimulated cortisol levels and psychotherapeutic outcome (Fischer & Cleare 2017). It is possible that methodological variations between studies may be accountable for the disparate results suggesting that cortisol levels during treatment may or may not be related to clinical outcome. For example, the distinction between unstimulated (basal, endogenous) and stimulated (in response to the intervention) cortisol levels has not been taken into account across studies. Indeed, Lass-Hennemann and Michael measured basal endogenous cortisol concentrations in patients with arachnophobia on the same day as the first

exposure session, whilst Meuret and Siegmund at their colleagues examined average stimulated cortisol release during the CBT intervention. Additionally, these studies have also varied in their methods used to calculate cortisol levels. Meuret et al. and Lass-Hennemann et al. analysed saliva samples, whilst Siegmund et al. collected and analysed blood serum samples (Lass-Hennemann & Michael 2014; Meuret et al. 2015; Siegmund et al. 2011). Given that changes in binding globulin concentrations, particularly cortisol-binding globulin, greatly affect total, but not free, serum cortisol levels, it is especially important to measure circulating free cortisol, rather than the total concentration (Coolens et al. 1987). Whilst salivary cortisol has been widely accepted to represent a surrogate marker for free serum cortisol, and is commonly used in psychological and stress research (Hellhammer et al. 2009), blood cortisol analysis is thought to provide a measurement of total (bound and unbound) cortisol concentration. Additionally, total cortisol measurements are also thought to provide an underestimate of the cortisol response during stress, because they cannot account for the exponential rise of free serum cortisol due to cortisol-binding globulin saturation (Vogeser et al. 2002). In summary, given the disparate methods used and the conflicting results obtained from these studies, it is difficult to obtain a clear picture regarding the relationship between endogenous glucocorticoid levels during treatment and therapeutic outcome. More independent studies with larger sample sizes and homogenous methodological procedures are necessary to shed light on the procedural elements responsible for the contrasting findings of previous works.

15.3 Predictive value of salivary cortisol and cortisone rises on the morning of treatment for clinical recovery

The cortisol awakening response (CAR), which can be defined as cortisol secretory activity in the first 45-60 minutes post-awakening, has attracted considerable attention as a biomarker of stress system activity that is distinct from diurnal variation. The CAR provides an alternative measure of HPA axis activity prior to exposure, that may have relevance to treatment outcome in anxiety disorders (Clow et al. 2004). Results from Meuret et al. demonstrated that a greater CAR was associated with enhanced clinical outcomes two months after treatment (Meuret et al. 2015), whilst other studies have not been able to demonstrate an association between the CAR and treatment efficacy (Brand et al. 2011; Dierckx et al. 2012). In accordance with previous nil findings, our results also

indicated that morning rises in salivary cortisol or cortisone levels were not predictive of improved clinical efficacy during the month after the CBT intervention.

In addition to the conflicting results regarding a potential relationship between the CAR and anxiety disorder treatment outcome, there is also considerable variation in normative CAR measurements obtained from healthy adult populations. Undoubtedly, a key reason why such discrepancies occur is that the CAR is sensitive to a range of confounding factors including: gender, age, smoking, awakening time, effect of light, participant adherence and weekday versus weekend collection (Clow et al. 2004). Given that all testing was conducted during the week and that smoking was an exclusion criterion in this study, these two potential confounders are unlikely to have impacted our results. Participant adherence problems are of particular importance in this study given that morning saliva sampling was carried out by the participants in their own homes. However, we tried to minimise this problem in several ways. Firstly, participants were given careful verbal and written instructions at the end of the screening session and were emphasised the importance of taking the samples at the correct times. Secondly, participants were handed the materials for saliva sampling in mock MEMS containers (which they thought to be real). Hence, the subjects were under the impression that the times at which they opened the containers was being recorded by the investigator. Despite this, it is possible that not all participants adhered exactly to the instructions, and given that the MEMS containers were not real, there was no way of verifying at which times the participants took their samples. Unfortunately, the CAR is especially sensitive to deviation from instructions. Given that the rate of increase in salivary cortisol concentration is often more than 100% within just 30 minutes of awakening, even minor deviations in the specific times that participants collected their morning samples (awakening, 30 minutes, 45 minutes) could have had substantial consequences on the values obtained. In contrast, small deviations in adherence to study protocol have been shown to be much less problematic in the evening or night when natural fluctuations in cortisol concentrations are far less marked (Clow et al. 2004).

15.4 Predictive value of baseline hair cortisol and cortisone for the clinical efficacy of one-session CBT

Our results indicated that baseline endogenous hair cortisol levels did not predict clinical symptomatic recovery at one-month follow-up. There was a trend for baseline hair cortisone to predict FSQ score change differently in either the placebo or hydrocortisone group. Further analysis revealed a non-significant trend for lower baseline hair cortisone to predict improved FSQ scores in the drug, but not in the placebo group (Figure 10). This finding suggests that a lower baseline hair cortisone level predicted enhanced clinical outcome in individuals who received hydrocortisone, or that individuals in the drug group with lower baseline hair cortisone concentration benefitted the most from exogenous hydrocortisone addition.

15.5 Predictive value of baseline hair cortisol and cortisone for the magnitude of change in fear bias

Results showed that baseline hair cortisol did not predict change in the fear bias at one-day follow-up in either the hydrocortisone or placebo group. However, baseline hair cortisone concentration significantly predicted change in fear bias across the groups. It appeared that there was a trend for this relationship to be stronger in one of the groups and further analysis revealed that in the drug group, lower baseline cortisone predicted stronger change in the fear bias in the 24-hour period after treatment (Figure 11).

A major limitation of current CBT initiatives is their inefficacy in a sub-group of patients. The ability to distinguish pre-treatment features that delineate patients who are likely to respond from those who are not, has the potential to maximise efficacy across the entire patient cohort. Currently, the most reliable predictor of the non-response phenomenon is the severity of the anxiety disorder and its co-morbidity with other mental health conditions, particularly personality disorders. However, markers that reflect the mechanism underlying effective treatment response are desirable. Our results indicate that baseline hair cortisone can significantly predict change in fear bias, an interface that has previously been shown represent a surrogate marker for the efficacy of interventional approaches in anxiety disorders (Reinecke, Waldenmaier, et al. 2013; Mathews & MacLeod 2005). Therefore, it is possible that endogenous hair cortisone concentration may represent a potential marker of response to CBT in individuals with

anxiety disorders. Future studies would do well to further explore this interaction, as finding predictors of the non-response phenomenon would allow therapy to be tailored to the individual whilst simultaneously guiding the development of novel interventions.

To the best of our knowledge, this is the first study to examine the predictive value of baseline endogenous hair cortisol and cortisone concentrations for the magnitude of change in fear bias on the day following a brief CBT session. The rationale behind this particular hypothesis was that previous studies have demonstrated that higher baseline endogenous cortisol levels are associated with lower attentional bias towards masked and unmasked stimuli in healthy participants (Van Honk et al. 1998; Van Honk et al. 2000). However, given the scarcity of research concerning how endogenous glucocorticoid functioning affects the fear bias in anxious individuals, we cannot confirm whether our results support or reject the previous literature. Future studies would do well to test the same hypothesis in a larger sample size, such that all statistical analyses can be better powered after the removal of outliers.

15.6 Methods of cortisol and cortisone analysis

Our salivary and hair cortisol and cortisone analyses were undertaken using LC-MS/MS. This technique affords high specificity and sensitivity, which was especially important in this study because the concentrations of cortisol and cortisol were low, and the two molecules are structurally very similar (Perogamvros et al. 2010). In previous works in which LC-MS/MS has not been used, the structural similarity of cortisol and cortisone has led to significant inter-assay variability. Indeed, some previous studies using cortisol immunoassays have in fact largely been measuring cortisone instead (Roberts & Roberts 2004). This is particularly important because compared to cortisol, cortisone levels are significantly higher in saliva because the parotid salivary gland tissue contains the enzyme hydroxysteroid dehydrogenase which converts cortisol to cortisone (Tomlinson & Stewart 2001).

15.7 Advantages and limitations of hair glucocorticoid analysis

In the vast majority of previous human psychoneuroendocrine research, endogenous cortisol levels have been measured from plasma, salivary or urinary samples. Whilst these methods are well-established, they reflect only acute circulating cortisol levels (plasma and saliva) or cumulative cortisol secretion over 12 or 24 hours (urine) (Stalder

et al. 2012). Given that the HPA axis is a highly reactive circadian system susceptible to a range of confounding factors, it is logical to hypothesise that single cortisol measurements are heavily affected by situational context and thus cannot inform about long-term secretion. This theory is supported by various findings revealing only weak to moderate test-retest associations for cortisol assessments obtained using these methods. Considering that salivary, urine and blood cortisol analyses cannot inform about cumulative cortisol secretion over longer periods of time, the analysis of hair cortisol concentration represents a major recent methodological advancement in this domain. The validity of hair cortisol as a retrospective index of long-term cortisol secretion has now been supported by research using a range of different paradigms in humans (e.g. Sauvé et al. 2007; Manenschijn et al. 2011; D'Anna-Hernandez et al. 2011; Kirschbaum et al. 2009) and two independent studies have provided strong evidence for high levels of intra-individual stability in hair cortisol concentration (Stalder et al. 2012). Finally, hair sampling presents several other advantages over urinary, salivary and blood analyses, in terms of the ease of obtaining samples, ability to store the samples at room temperature and resistance to participant non-adherence regarding when the samples are collected.

On the other hand, certain methodological challenges concerning hair cortisol analysis must also be acknowledged. Most authors assume that hair cortisol is representative of systemic concentrations. However, it is possible that local cortisol production may also be measured in hair analyses, given evidence to suggest that hair follicles contain a functional equivalent of the HPA axis which is capable of synthesising cortisol following stimulation by corticotrophin-releasing hormone (Ito 2005). Further, the method by which cortisol enters the hair is currently unknown, although it is highly likely to be passive diffusion from the blood. In this scenario, hair cortisol would reflect the integrated free cortisol fraction, rather than the total cortisol concentration in the serum (Russell et al. 2012). Additionally, various possible confounding factors may also influence hair cortisol measurements. For example, it is possible that cortisol present in sebaceous and eccrine secretions may coat the outer hair cuticle (Pragst & Balikova 2006; Raul et al. 2004). Thus, hair washing frequency or the extent to which subjects have sweated on the back of their head may influence cortisol measurements, although no studies to date have been conducted to confirm that cortisol is even present in sebum or sweat. Moreover, the effect of natural hair colour or artificial hair

treatments on hair cortisol concentration is another topic of debate. Whilst studies in humans have not been able to detect a significant effect of natural hair pigmentation on cortisol levels (Sauvé et al. 2007; Manenschijn et al. 2011), a study in dogs revealed that hair melanin content (thus pigment) could impact how cortisol is sequestered into hair, thereby affecting overall hair cortisol concentration. In terms of cosmetic hair treatment, it has previously been demonstrated that artificial hair colouration significantly decreases cortisol levels compared to controls (Sauvé et al. 2007). Putative mechanisms for this finding include the hypothesis that cosmetic treatments such as bleaching may increase the porosity of hair, leading to greater cortisol leaching out of the hair (Boumba et al. 2006), or that such treatments add weight to the hair causing a dilution-like effect.

15.8 Study strengths and limitations

This study presents several advantages. Firstly, in addition to cortisol measurements we also analysed cortisone concentration in hair and saliva. This was in light of recent evidence suggesting that salivary cortisone may provide a more detailed and robust reflection of systemic cortisol concentration than salivary cortisol (Perogamvros et al. 2010). Moreover, obtaining glucocorticoid measurements from hair samples provided us with an index of cumulative cortisol secretion over a period of months, which compensated for a limitation of analysing saliva samples which only provided measurements of acute circulating glucocorticoid levels. Analysis by LC-MS/MS also granted high specificity and sensitivity for reliable and accurate cortisol and cortisone analysis. Finally, by obtaining measurements of salivary cortisol on the morning of the therapy session, prior to the stress-inducing exposure tasks and also during the CBT, we were able to analyse the cortisol awakening response, stimulated and hydrocortisone-induced cortisol release.

In addition to the limitations mentioned in chapter 2, notably the small sample size, the fact that only 30.3% of the participant cohort had a spider phobia and the possibility that not all participant fully engaged in the CBE, certain limitations arising in this study also warrant attention. Given that one of the aims of the study was to assess salivary cortisol and cortisone release in a naturalistic environment, tight control of a plethora of possible confounds was not possible. Some of these confounds might include: potential variability in the exact times that participants collected saliva samples when unaccompanied at

home, sleep-related factors, smoking, alcohol, caffeine, central nervous system stimulant intake prior to exposure (albeit discouraged), food consumption prior to testing and circadian rhythm. An additional list of confounding variables that may have impacted hair cortisol measurements also deserve mention. These include: hair washing frequency, natural hair colour and artificial hair treatments. Furthermore, as previously mentioned, it is possible that CBT did not occur at peak drug level, given that complete hydrocortisone absorption appeared to take longer than our estimates of one hour. Finally, our data cannot distinguish whether endogenous salivary and hair cortisol and cortisone were active agents in shaping treatment outcome, or whether they simply reflected a distal biomarker for another factor related to treatment response.

15.9 Future directions

As mentioned above, this study does not delineate whether salivary and hair cortisol and cortisone were active agents modulating treatment or were simply biomarkers of treatment response. If the former is correct, then exploration into ways in which endogenous glucocorticoids can be manipulated to naturally facilitate extinction is warranted. If the latter, then cortisol and cortisone measures may require further exploration as biomarkers that could be used to guide the development of improved treatments for anxiety disorders, and to help determine which individuals will likely respond to specific interventional techniques such as exposure. Future studies are also needed to address questions regarding optimal timing of CBT administration, given that salivary cortisol and cortisone continued to rise one hour after drug administration in this study.

Additionally, there remain many outstanding questions regarding hair cortisol analysis. These include mechanisms of cortisol incorporation into the hair, the extent to which hair cortisol originates from blood, eccrine and/or sebaceous sources, and if hair cortisol reflects total and/or free system cortisol. Secondly, future studies would do well to assess the impact of hair washing, artificial treatments, ethnicity, age, hair colour, sex and seasonal influences on hair cortisol concentration. Once these initial questions are answered, it is likely that hair cortisol analysis will be adopted as an indispensable tool for measuring chronic stress and long-term cumulative cortisol secretion in a plethora of clinical and experimental settings. Finally, the majority of previous human studies using hair cortisol analysis as a chronic biomarker of stress are associative thus cannot confirm

causation of the stress levels. Moving forward, a greater emphasis on long-term interventional studies that shed light on the factors that initiate and control cortisol release into the hair, will enhance the utility of this novel biomarker.

16. Conclusions

Overall, hydrocortisone administration caused a significant increase in salivary cortisol and cortisone concentrations. Salivary cortisol and cortisone measurements both during treatment and of the morning of treatment were not predictive of clinical symptomatic improvement or the magnitude of change in fear bias. Baseline endogenous hair cortisol levels were not predictive of enhanced clinical outcome during the month following treatment, or greater changes in the fear bias on the day after treatment. Baseline hair cortisone also did not predict clinical recovery at one-month follow-up, but there was a non-significant trend for lower endogenous hair cortisone to predict improved FSQ score change in individuals receiving hydrocortisone. Finally, baseline hair cortisone measurements significantly predicted change in fear bias across the groups. There was a trend for this prediction to be stronger in the drug group, with lower baseline cortisone predicting stronger change in the fear bias in the 24 hour period after treatment.

This study provides insight into the interaction between the endogenous glucocorticoid system, exogenous hydrocortisone administration and the mechanisms and trajectories of clinical symptomatic change in a cohort of individuals with high spider fear receiving a single session of exposure-based CBT. It also sheds light on the potential for baseline endogenous hair cortisone concentration to be used to distinguish individuals who are likely to respond to therapy from those who are not. The major advantage of this study is the analysis of long-term, cumulative measures of cortisol and cortisone secretion from hair samples, which compensates for a limitation of previous studies which have predominantly analysed short-term cortisol measures obtained from blood, saliva or urine. Finally, several limitations and confounding factors that must be considered when interpreting the results of this study have been detailed herein, and future studies would benefit from addressing the points raised.

Chapter 4 – Overall discussion and conclusions

This study had three primary aims: i) to investigate the potential of hydrocortisone to enhance the efficacy of one-session, computer-based CBT in in a cohort of individuals with high spider fear, ii) to determine whether early alterations to the fear bias on the day after treatment predicted glucocorticoid-enhanced outcomes at one-month follow-up and iii) to evaluate the predictive value of hair and salivary cortisol and cortisone concentrations prior to and during treatment for early changes in fear bias and for clinical improvement at one month.

Our results revealed that hydrocortisone did not improve clinical, behavioural or cognitive bias measures as compared to placebo. Early changes in the fear bias on the day after treatment were not predictive of clinical symptomatic improvement one-month later in either the hydrocortisone or placebo group. Hydrocortisone administration resulted in a significant acute increase in salivary cortisol and cortisone. Endogenous salivary cortisol and cortisone levels during treatment and on the morning of treatment were not predictive of symptomatic improvement. Baseline endogenous hair cortisol levels were also not predictive of improved clinical outcome or early fear bias changes. Endogenous hair cortisone significantly predicted change in fear bias across the groups and there was a trend for this to be stronger in one of the groups, such that lower baseline hair cortisone significantly predicted greater fear bias change in the hydrocortisone but not the placebo group. Finally, there was a non-significant trend for lower hair cortisone to predict enhanced clinical outcome in the drug group, but not in those who received placebo.

Although the strengths and limitations of this study have been detailed in the body above, those that are particularly pertinent to our work will be reinforced below. The major advantages of this study are: i) the use of behavioural outcome measures in addition to clinical (self-reported) measures and ii) analysis of hair samples which provides an indication of long-term, cumulative cortisol and cortisone secretion, thereby compensating for one of the limitations of salivary analysis. Certain key limitations also warrant further discussion. Primarily, only 30.3% participants fulfilled the criteria for a specific phobia (DSM-V) and it is possible that the overall spider fear intensity was too

low for the additional effects of hydrocortisone to reach significance. Further, due to the small sample size, the generalisability of our findings is potentially limited. Participant non-adherence could also represent a problem in this study. It is possible that not all participants fully engaged in the CBE task and/or did not adhere to the timing protocol for collecting saliva samples at home. Furthermore, our data cannot distinguish whether the across-group clinical symptomatic improvement seen at one-month was due to the CBT intervention or some other factor, given that a control arm of participants who did not receive CBT treatment was not included in this study.

Numerous future directions have also been addressed above. The most important aim for future work is to conduct similar studies in a population of clinically-diagnosed individuals. Further, given that our study applied extremely stringent inclusion criteria (due to the plethora of factors known to influence endogenous glucocorticoid levels e.g. age, pregnancy, medication), further studies in less restrictive and larger samples are needed to confirm the generalisability of our findings. Finally, the results of this study suggest that the CBT intervention may not have been delivered at drug peak level. Future studies therefore also need to address the protocol for the optimum timing of CBT delivery, which, if performed at drug peak, could lead to significant group differences in clinical outcomes.

Overall, this study has provided insight into the relationships between the endogenous glucocorticoid system, exogenous hydrocortisone administration, and the mechanisms and trajectories of change following a brief session of computer-based CBT in individuals with high spider fear. Further research is now required to pinpoint exactly which factors have previously underpinned the hydrocortisone augmentation effect seen in other studies employing exposure-based CBT for anxiety disorders. An improved understanding of the mechanisms driving enhanced treatment outcomes will better inform the design of future studies and will ultimately guide the development of improved psychological and psychotherapeutic tools for more effective treatment of anxiety disorders in the future.

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Appendices

SAS - 1

The following four statements are related to your relationship towards spiders. For each statement, please indicate to what degree it applies to you. Please evaluate each statement by choosing a number between 0 (does not apply at all) to 6 (does apply very much). Please respond quickly and without thinking about your response too long. There are no right or wrong answers. We are interested in your individual opinion. Please remember to answer ALL statements.

	not at all						very much
	0	1	2	3	4	5	6
1. **I am afraid of spiders.**							
2. **When seeing a spider I become very nervous and my heart beats faster.**							
3. **I avoid spiders.**							
4. **My fear of spiders burdens me.**							

Appendix A. Spider anxiety screening (SAS) (Rinck et al. 2002)**.**

ACS

A number of statements are written below. Read each statement and then circle the appropriate number to the right of the statement to indicate how often it described you.

	Almost Never	Sometimes	Often	Frequently
1. It's very hard for me to concentrate on a difficult task when there are noises around	1	2	3	4
2. When I need to concentrate and solve a problem, I have trouble focusing my attention	1	2	3	4
3. When I am working hard on something, I still get distracted by events around me	1	2	3	4
4. My concentration is good even if there is music in the room around me	1	2	3	4
5. When concentrating, I can focus my attention so that I become unaware of what's going on in the room around me	1	2	3	4
6. When I am reading or studying, I am easily distracted if there are people talking in the same room	1	2	3	4
7. When trying to focus my attention on something, I have difficulty blocking out distracting thoughts	1	2	3	4
8. I have a hard time concentrating when I'm excited about something	1	2	3	4
9. When concentrating I ignore feelings of hunger or thirst	1	2	3	4

10. I can quickly switch from one task to another	1	2	3	4
11. It takes me a while to get really involved in a new task	1	2	3	4
12. It is difficult for me to coordinate my attention between the listening and writing required when taking notes during lectures	1	2	3	4
13. I can become interested in a new topic very quickly when I need to	1	2	3	4
14. It is easy for me to read or write while I'm also talking on the phone.	1	2	3	4
15. I have trouble carrying on two conversations at once	1	2	3	4
16. I have a hard time coming up with new ideas quickly	1	2	3	4
17. After being interrupted or distracted, I can easily shift my attention back to what I was doing before	1	2	3	4
18. When a distracting thought comes to mind, it is easy for me to shift my attention away from it	1	2	3	4
19. It is easy for me to alternate between two different tasks	1	2	3	4
20. It is hard for me to break from one way of thinking about something and look at it from another point of view	1	2	3	4

Appendix B. Attentional Control Scale (ACS) (Derryberry & Reed 2002)**.**

ASI

Please indicate how well each statement applies to you.

		Very little	A little	some	much	Very much
1	When I feel like I'm not getting enough air I get scared that I might suffocate	0	1	2	3	4
2	Smothering sensations scare me	0	1	2	3	4
3	It scares me when I become short of breath	0	1	2	3	4
4	When my chest feels tight, I get scared that I won't be able to breathe properly	0	1	2	3	4
5	It scares me when I feel faint	0	1	2	3	4
6	When my throat feels tight, I worry that I could choke to death	0	1	2	3	4
7	It scares me when my heart beats rapidly	0	1	2	3	4
8	When my breathing becomes irregular, I fear that something bad will happen	0	1	2	3	4
9	It scares me when I feel "shaky" (trembling)	0	1	2	3	4
10	When I have trouble swallowing, I worry that I could choke	0	1	2	3	4
11	It frightens me when my surroundings seem strange or unreal	0	1	2	3	4
12	It scares me when my body feels strange or different in some way	0	1	2	3	4
13	It is important for me not to appear nervous	0	1	2	3	4
14	I believe it would be awful to vomit in public	0	1	2	3	4
15	I think it would be horrible for me to faint in public	0	1	2	3	4
16	I worry that other people will notice my anxiety	0	1	2	3	4
17	When I tremble in the presence of others I fear what people might think of me	0	1	2	3	4
18	When I begin to sweat in a social situation, I fear people will think negatively of me	0	1	2	3	4

19	It scares me when I blush in front of people	0	1	2	3	4
20	When I feel a strong pain in my stomach, I worry it could be cancer	0	1	2	3	4
21	When my head is pounding I worry I could have a stroke	0	1	2	3	4
22	When my heart is beating rapidly, I worry that I might have a heart attack	0	1	2	3	4
23	When my face feels numb, I worry that I might be having a stroke	0	1	2	3	4
24	When I feel pain in my chest, I worry that I'm going to have a heart attack	0	1	2	3	4
25	When I feel dizzy, I worry there is something wrong with my brain	0	1	2	3	4
26	When my stomach is upset, I worry that I might be seriously ill	0	1	2	3	4
27	When I notice my heart skipping a beat, I worry that something might be seriously wrong with me	0	1	2	3	4
28	When I get diarrhoea, I worry that I might have something wrong with me	0	1	2	3	4
29	It scares me when I am nauseous	0	1	2	3	4
30	It scares me when I feel tingling or prickling sensations in my hands	0	1	2	3	4
31	When I feel "spacey" or spaced out I worry that I may be mentally ill	0	1	2	3	4
32	When my thoughts seem to speed up, I worry that I might be going crazy	0	1	2	3	4
33	When I have trouble thinking clearly, I worry there is something wrong with me	0	1	2	3	4
34	When I cannot keep my mind on a task, I worry that I might be going crazy	0	1	2	3	4
35	It scares me when I am unable to keep my mind on a task	0	1	2	3	4
36	When my mind goes blank I worry there is something terribly wrong with me	0	1	2	3	4

Appendix B. Anxiety Sensitivity Index (ASI) (Taylor 1998)**.**

BDI-II

Instructions

This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the one statement in each group that best describes the way you have been feeling during the past two weeks, including today. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all of the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism
0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure
0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure
0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

6. Punishment Feelings
0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike
0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness
0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal thoughts or Wishes
0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

5. Guilty Feelings

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

10. Crying

0 I don't cry any more than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.
2b My appetite is much greater than usual.

3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

15. Loss of Energy 0 I have as much energy as ever. 1 I have less energy than I used to have. 2 I don't have enough energy to do very much. 3 I don't have enough energy to do anything. **16. Changes in Sleeping Pattern** 0 I have not experienced any change in my sleeping pattern 1a I sleep somewhat more than usual 1b I sleep somewhat less than usual. 2a I sleep a lot more than usual. 2b I sleep a lot less than usual. 3a I sleep most of the day. 3b I wake up 1-2 hours early and can't get back to sleep.	**21. Loss of Interest in Sex** 0 I have not noticed any recent change in my interest in sex. 1 I am less interested in sex than I used to be. 2 I am much less interested in sex now. 3 I have lost interest in sex completely.

Appendix B. Beck Depression Inventory (BDI). (Beck et al. 1996)**.**

BIS and BAS

Please indicate to what degree you agree or disagree with the following statements.

		Strongly agree	Slightly agree	Slightly disagree	Strongly disagree
1	If I think something unpleasant is going to happen I usually get pretty "worked up."	1	2	3	4
2	I worry about making mistakes.	1	2	3	4
3	Criticism or scolding hurts me quite a bit.	1	2	3	4
4	I feel pretty worried or upset when I think or know somebody is angry at me.	1	2	3	4
5	Even if something bad is about to happen to me, I rarely experience fear or nervousness.	1	2	3	4
6	I feel worried when I think I have done poorly at something.	1	2	3	4
7	I have very few fears compared to my friends.	1	2	3	4
8	When I get something I want, I feel excited and energized.	1	2	3	4
9	When I'm doing well at something, I love to keep at it.	1	2	3	4
10	When good things happen to me, it affects me strongly.	1	2	3	4
11	It would excite me to win a contest.	1	2	3	4
12	When I see an opportunity for something I like, I get excited right away.	1	2	3	4
13	When I want something, I usually go all-out to get it.	1	2	3	4
14	I go out of my way to get things I want.	1	2	3	4
15	If I see a chance to get something I want, I move on it right away.	1	2	3	4
16	When I go after something I use a "no holds barred" approach.	1	2	3	4

17	I will often do things for no other reason than that they might be fun.	1	2	3	4
18	I crave excitement and new sensations.	1	2	3	4
19	I'm always willing to try something new if I think it will be fun.	1	2	3	4
20	I often act on the spur of the moment.	1	2	3	4

Appendix B. Behavioural Inhibition Scale (BIS) and Behavioural Activation Scale (BAS) (Carver & White 1994)**.**

E.P.Q. (Adult)

INSTRUCTIONS Please answer each question by putting a circle around the "YES" or the "NO" following the question. There are no right or wrong answers, and no trick questions. Work quickly and do not think too long about the exact meaning of the questions.

PLEASE REMEMBER TO ANSWER EACH QUESTION

1. Do you have many different hobbies?	YES	NO
2. Do you stop to think things over before doing anything?	YES	NO
3. Does your mood often go up and down?	YES	NO
4. Have you ever taken the praise for something you knew someone else had really done?	YES	NO
5. Are you a talkative person?	YES	NO
6. Would you being in debt worry you?	YES	NO
7. Do you ever feel "just miserable" for no reason?	YES	NO
8. Were you ever greedy by helping yourself to more than your share of anything?	YES	NO
9. Do you lock up your house carefully at night?	YES	NO
10. Are you rather lively?	YES	NO
11. Would it upset you a lot to see a child or an animal suffer?	YES	NO
12. Do you often worry about things you should not have done or said?	YES	NO
13. If you say you will do something, do you always keep your promise no matter how inconvenient it might be?	YES	NO
14. Can you usually let yourself go and enjoy yourself at a lively party?	YES	NO
15. Are you an irritable person?	YES	NO
16. Have you ever blamed someone for doing something you knew was really your fault?	YES	NO
17. Do you enjoy meeting new people?	YES	NO
18. Do you believe insurance schemes are a good idea?	YES	NO
19. Are your feelings easily hurt?	YES	NO
20. Are *all* your habits good and desirable ones?	YES	NO
21. Do you tend to keep in the background on social occasions?	YES	NO
22. Would you take drugs which may have strange or dangerous effects?	YES	NO

23. Do you often feel “fed-up”?	YES	NO
24. Have you ever taken anything (even a pin or button) that belonged to someone else?	YES	NO
25. Do you like going out a lot?	YES	NO
26. Do you enjoy hurting people you love?	YES	NO
27. Are you often troubled about feelings of guilt?	YES	NO
28. Do you sometimes talk about things you know nothing about?	YES	NO
29. Do you prefer reading to meeting people?	YES	NO
30. Do you have enemies who want to harm you?	YES	NO
31. Would you call yourself a nervous person?	YES	NO
32. Do you have many friends?	YES	NO
33. Do you enjoy practical jokes that can sometimes really hurt people?	YES	NO
34. Are you a worrier?	YES	NO
35. As a child did you do as you were told immediately and without grumbling?	YES	NO
36. Would you call yourself happy-go-lucky?	YES	NO
37. Do good manners and cleanliness matter much to you?	YES	NO
38. Do you worry about awful things that might happen?	YES	NO
39. Have you ever broken or lost something belonging to someone else?	YES	NO
40. Do you usually take the initiative in making new friends?	YES	NO
41. Would you call yourself tense or “highly-strung”?	YES	NO
42. Are you mostly quiet when you are with other people?	YES	NO
43. Do you think marriage is old-fashioned and should be done away with?	YES	NO
44. Do you sometimes boast a little?	YES	NO
45. Can you easily get some life into a rather dull party?	YES	NO
46. Do people who drive carefully annoy you?	YES	NO
47. Do you worry about your health?	YES	NO
48. Have you ever said anything bad or nasty about anyone?	YES	NO
49. Do you like telling jokes and funny stories to your friends?	YES	NO
50. Do most things taste the same to you?	YES	NO
51. As a child were you ever cheeky to your parents?	YES	NO
52. Do you like mixing with people?	YES	NO

53. Does it worry you if you know there are mistakes in your work? YES NO
54. Do you suffer from sleeplessness? YES NO
55. Do you always wash before a meal? YES NO
56. Do you nearly always have a “ready answer” when people talk to you? YES NO
57. Do you like to arrive at appointments in plenty of time? YES NO
58. Have you often felt listless and tired for no reason? YES NO
59. Have you ever cheated at a game? YES NO
60. Do you like doing things in which you have to act quickly? YES NO
61. Is (or was) your mother a good woman? YES NO
62. Do you often feel life is very dull? YES NO
63. Have you ever taken advantage of someone? YES NO
64. Do you often take on more activities than you have time for? YES NO
65. Are there several people who keep trying to avoid you? YES NO
66. Do you worry a lot about your looks? YES NO
67. Do you think people spend too much time safeguarding their future with savings and insurances? YES NO
68. Have you ever wished that you were dead? YES NO
69. Would you dodge paying taxes if you were sure you could never be found out? YES NO
70. Can you get a party going? YES NO
71. Do you try not to be rude to people? YES NO
72. Do you worry too long after an embarrassing experience? YES NO
73. Have you ever insisted on having your own way? YES NO
74. When you catch a train do you often arrive at the last minute? YES NO
75. Do you suffer from “nerves”? YES NO
76. Do your friendships break up easily without it being your fault? YES NO
77. Do you often feel lonely? YES NO
78. Do you always practice what you preach? YES NO
79. Do you sometimes like teasing animals? YES NO
80. Are you easily hurt when people find fault with you or the work you do? YES NO
81. Have you ever been late for an appointment or work? YES NO

82. Do you like plenty of bustle and excitement around you?	YES	NO
83. Would you like other people to be afraid of you?	YES	NO
84. Are you sometimes bubbling over with energy and sometimes very sluggish?	YES	NO
85. Do you sometimes put off until tomorrow what you ought to do today?	YES	NO
86. Do other people think of you as being very lively?	YES	NO
87. Do people tell you a lot of lies?	YES	NO
88. Are you touchy about some things?	YES	NO
89. Are you always willing to admit it when you have made a mistake?	YES	NO
90. Would you feel very sorry for an animal caught in a trap?	YES	NO

PLEASE CHECK TO SEE THAT YOU HAVE ANSWERED ALL THE QUESTIONS

Appendix B. Eysenck Personality Questionnaires (EPQ). (Eysenck & Eysenck 1994)**.**

PSRS (23-item)

Instructions: This questionnaire asks about your reactions to situations that you may have experienced in the past. Three answers are suggested. Please indicate the answer that most closely describes your own reaction in general. Please don't skip any item, even if it may be hard to find the best answer.

01 When tasks and duties build up to the extent that they are hard to manage . . .
- **O** I am generally untroubled
- **O** I usually feel a little uneasy
- **O** I normally get quite nervous

02 When I want to relax after a hard day at work . . .
- **O** This is usually quite difficult for me
- **O** I usually succeed
- **O** I generally have no problem at all

03 When I have conflicts with others that may not be immediately resolved . . .
- **O** I generally shrug it off
- **O** It usually affects me a little
- **O** It usually affects me a lot

04 When I make a mistake . . .
- **O** In general, I remain confident
- **O** I sometimes feel unsure about my abilities
- **O** I often have doubts about my abilities

05 When I'm wrongly criticized by others . . .
- **O** I am normally annoyed for a long time
- **O** I am annoyed for just a short time
- **O** In general, I am hardly annoyed at all

06 When I argue with other people . . .
- **O** I usually calm down quickly
- **O** I usually stay upset for some time
- **O** It usually takes me a long time until I calm down

07 When I have little time for a job to be done . . .
- **O** I usually stay calm
- **O** I usually feel uneasy
- **O** I usually get quite agitated

08 When I make a mistake . . .
- **O** I am normally annoyed for a long time
- **O** I am normally annoyed for a while
- **O** I generally get over it easily

09 When I am unsure what to do or say in a social situation . . .

- **O** I generally stay cool
- **O** I often feel warm
- **O** I often begin to sweat

10 When I have spare time after working hard . . .

- **O** It often is difficult for me to unwind and relax
- **O** I usually need some time to unwind properly
- **O** I am usually able to unwind effectively and forget about the problems of the day

11 When I am criticized by others . . .

- **O** Important arguments usually come to my mind when it is too late to still make my point
- **O** I often have difficulty finding a good reply
- **O** I usually think of a reply to defend myself

12 When something does not go the way I expected . . .

- **O** I usually stay calm
- **O** I often get uneasy
- **O** I usually get very agitated

13 When I do not attain a goal . . .

- **O** I usually remain annoyed for a long time
- **O** I am usually disappointed, but recover soon
- **O** In general, I am hardly concerned at all

14 When others criticize me . . .

- **O** I generally don't lose confidence at all
- **O** I generally lose a little confidence
- **O** I generally feel very unconfident

15 When I fail at something . . .

- **O** I usually find it hard to accept
- **O** I usually accept it to some degree
- **O** In general, I hardly think about it

16 When there are too many demands on me at the same time . . .

- **O** I generally stay calm and do one thing after the other
- **O** I usually get uneasy
- **O** Usually, even minor interruptions irritate me

17 When others say something incorrect about me . . .

- **O** I usually get quite upset
- **O** I normally get a little bit upset
- **O** In general, I shrug it off

18 When I fail at a task . . .

- **O** I usually feel very uncomfortable
- **O** I usually feel somewhat uncomfortable
- **O** In general, I don't mind

19 When I argue with others . . .

- **O** I usually get very upset
- **O** I usually get a little bit upset
- **O** I usually don't get upset

20 When I am under stress . . .

- **O** I usually can't enjoy my leisure time at all
- **O** I usually have difficulty enjoying my leisure time
- **O** I usually enjoy my leisure time

21 When tasks and duties accumulate to the extent that they are hard to cope with . . .

- **O** My sleep is unaffected
- **O** My sleep is slightly disturbed
- **O** My sleep is very disturbed

22 When I have to speak in front of other people . . .

- **O** I often get very nervous
- **O** I often get somewhat nervous
- **O** In general, I stay calm

23 When I have many tasks and duties to fulfill . . .

- **O** In general, I stay calm
- **O** I usually get impatient
- **O** I often get irritable

End of questionnaire – thank you!

Appendix B. Perceived Stress Reactivity Scale (PSRS) (Schlotz et al. 2011)**.**

TRAIT QUESTIONNAIRE

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you GENERALLY feel. There are no right and wrong answers. Do not spend too much time on each statement but give the answer which seems to describe you best.

	Not at all	Somewhat	Moderately	Very much
1. I feel pleasant	1	2	3	4
2. I feel nervous and restless	1	2	3	4
3. I feel satisfied with myself	1	2	3	4
4. I wish I could be as happy as others seem to be	1	2	3	4
5. I feel like a failure	1	2	3	4
6. I feel rested	1	2	3	4
7. I am 'cool, calm and collected'	1	2	3	4
8. I feel that the difficulties are piling up so that I cannot overcome them	1	2	3	4
9. I worry too much over something that doesn't really matter	1	2	3	4
10. I am happy	1	2	3	4
11. I have disturbing thoughts	1	2	3	4
12. I lack self-confidence	1	2	3	4
13. I feel secure	1	2	3	4
14. I make decisions easily	1	2	3	4
15. I feel inadequate	1	2	3	4
16. I am content	1	2	3	4
17. Some unimportant thoughts run through my mind and bother me	1	2	3	4
18. I take disappointments so keenly				

that I can't put them out of my mind	1	2	3	4
19. I am a steady person	1	2	3	4
20. I get in a state of tension or turmoil as I think over recent concerns and interests	1	2	3	4

Appendix B. State-Trait Anxiety Inventory (STAI). (Spielberger 1983)**.**

Trauma History Checklist

Many people have lived through or witnessed a very stressful and traumatic event at some point in their lives.
Below is a sequence of descriptions of traumatic events. When you read an event that has happened to you, or you have witnessed, please circle Yes. Otherwise, please circle No.

Have you experienced or witnessed:

1. **A serious accident, fire, or explosion?** *(Eg. an industrial, farm, car, plane or boating accident.)* Yes / No

2. **A Natural disaster?** *(Eg. A tornado, flood or major earthquake)* Yes / No

3. **A Non-sexual assault by a family member or someone you know?** *(Eg. Being mugged, physically attacked, shot, stabbed or held at gunpoint)* Yes / No

4. **A Non-sexual assault by a stranger?** *(Eg. Being mugged, physically attacked, shot, stabbed or held at gunpoint)* Yes / No

5. **A sexual assault by a family member or someone you know?** *(Eg. rape or attempted rape)* Yes / No

6. **Sexual assault by a stranger?** *(Eg rape or attempted rape)* Yes / No

7. **Military combat or a war zone?** Yes / No

8. **Sexual contact when you were younger than 18 with someone who was 5 or more years older than you?** *(Eg. Contact with genitals, breasts)* Yes / No

9. **Imprisonment?** *(Eg. Prison inmate, prisoner of war, hostage)* Yes / No

10. **Torture?** Yes / No

11. **Life-threatening illness?** Yes / No

12. **Car crash?** Yes / No

13. **Any other traumatic event?** Yes / No
    Please specify:

Appendix B. Trauma history checklist (THC). (Holmes et al. 2004).

FSQ - 1

The following statements are related to your relationship towards spiders. For each statement, please indicate to what degree it applies to you. Please evaluate each statement by choosing a number between 0 (does not apply at all) to 6 (does apply very much). Please respond quickly and without thinking about your response too long. There are no right or wrong answers. We are interested in your individual opinion. Please remember to answer ALL statements.

not at all very much

		0	1	2	3	4	5	6
1	If I came across a spider now, I would get help from someone else to remove it.							
2	Currently, I am sometimes on the look out for spiders.							
3	If I saw a spider now, I would think it will harm me.							
4	I now think a lot about spiders.							
5	I would be somewhat afraid to enter a room now, where I have seen a spider before.							
6	I now would do anything to try to avoid a spider.							
7	Currently, I sometimes think about getting bit by a spider.							
8	If I encountered a spider now, I wouldn't be able to deal effectively with it.							
9	If I encountered a spider now, it would take a long time to get it out of my mind.							
10	If I came across a spider now, I would leave the room.							
11	If I saw a spider now, I would think it will try to jump on me.							
12	If I saw a spider now, I would ask someone else to kill it.							
13	If I encountered a spider now, I would have images of it trying to get me.							
14	If I saw a spider now I would be afraid of it.							
15	If I saw a spider now, I would feel very panicky.							
16	Spiders are one of my worst fears.							

17	I would feel very nervous if I saw a spider now.							
18	If I saw a spider now I would probably break out in a sweat and my heart would beat faster.							

Appendix C. Fear of Spiders Questionnaire (FSQ) (Szymanski & O'Donohue 1995)**.**

VAS

At the moment I feel:

ANXIOUS

not at all ◆——————————————————◆ extremely

SLEEPY

not at all ◆——————————————————◆ extremely

FLUSHED

not at all ◆——————————————————◆ extremely

TEARFUL

not at all ◆——————————————————◆ extremely

NAUSEOUS

not at all ◆——————————————————◆ extremely

HOPELESS

not at all ◆——————————————————◆ extremely

TREMOR

not at all ◆——————————————————◆ extremely

SAD

not at all ◆——————————————————◆ extremely

DIZZY

not at all ◆——————————————————◆ extremely

DEPRESSED

not at all ◆——————————————————◆ extremely

TACHYCARDIA

not at all ◆——————————————————————◆ extremely

ALERT

not at all ◆——————————————————————◆ extremely

Appendix D. Visual analogue scales for mood and physiological responses.